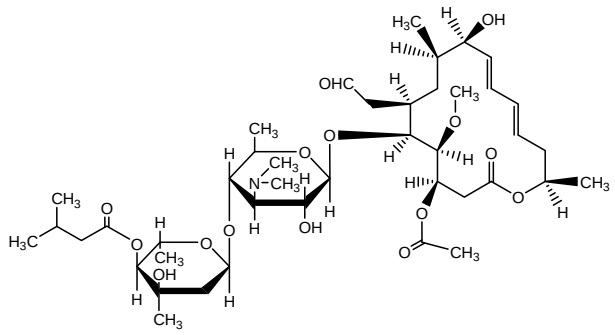
Josamycin



$C_{42}H_{69}NO_{15}$: 827.99

[(2*S*,3*S*,4*R*,6*S*)-6-[(2*R*,3*S*,4*R*,5*R*,6*S*)-6-[[(4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-4-Acetyloxy-10-hydroxy-5-methoxy-9,16-dimethyl-2-oxo-7-(2-oxoethyl)-1-oxacyclohexadeca-11,13-dien-6-yl]oxy]-4-(dimethylamino)-5-hydroxy-2-methyloxan-3-yl]oxy-4-hydroxy-2,4-dimethyloxan-3-yl] 3-methylbutanoate [*16846-24-5*]

Josamycin is a macrolide substance having antibacterial activity produced by the growth of *Streptomyces narboensis* var. *josamyceticus*.
Josamycin contains not less than 900 μg (potency) and not more than 1100 μg (potency) per mg of josamycin ($C_{42}H_{69}NO_{15}$: 827.99), calculated on the dried basis.

Description Josamycin appears as white to yellowish white powder.
Josamycin is very soluble in methanol or in ethanol (95), and very slightly soluble in water.

Identification (1) Determine the absorption spectra of solutions of Josamycin and Josamycin RS in methanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve separately 5 mg each of Josamycin and Josamycin RS in 1 mL of methanol, add diluted methanol (1 in 2) to make 100 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions: the retention time of josamycin from the test solution is the same as that of josamycin from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (2).

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Josamycin according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

(2) ***Related substances***—Dissolve 50 mg of Josamycin in 5 mL of methanol, add diluted methanol (1 in 2) to make 50 mL, and use this solution as the test solution. Perform the test with 10 μL of the test solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area. Calculate the amount of each peak other than josamycin by the area percentage method: not more than 6 %. The total area of the peaks other than josamycin is not more than 20 %.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 231 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 50 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: Dissolve 119 g of sodium perchlorate hydrate in water to make 1000 mL, and adjust the pH to 2.5 with 1 mol/L hydrochloric acid TS. To 600 mL of this solution add 400 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of josamycin is about 10 minutes.

System suitability

Test for required detectability: Pipet 3 mL of the test solution, add dilute methanol (1 in 2) to make exactly 50 mL, and use this solution as the system suitability solution. Pipet 2 mL of the system suitability solution, and add dilute methanol (1 in 2) to make exactly 20 mL. Confirm that the peak area of josamycin obtained from 10 μL of this solution is equivalent to 8 to 12 % of the peak area of josamycin from the system suitability solution.

System performance: Dissolve 0.05 g of Josamycin in 50 mL of 0.1 mol/L potassium dihydrogen phosphate TS (pH 2.0), and allow to stand at 40 °C for 3 hours. Adjust the pH of this solution to 6.8 to 7.2 with 2 mol/L sodium hydroxide TS, and add 50 mL of methanol. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peaks of josamycin and josamycin S_1, having the relative retention time of about 0.9 with respect to josamycin, is not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the peak areas of josamycin is not more than 1.5 %.

Time span of measurement: About 4 times as long as the retention time of josamycin beginning after the solvent peak

Loss on Drying Not more than 1.0 % (0.5, in vacuum, P_2O_5, 60 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay ***The Cylinder-plate method*** (1) Agar media for seed and base layer- Use the culture medium in I 2 1) (1) ① under Microbial Assay for Antibiotics.

(2) Test organism- *Bacillus subtilis* ATCC 6633

(3) Weigh accurately about 30 mg (potency) of Josamycin, dissolve in 5 mL of methanol, add sterile purified water to make exactly 100 mL, and use this solution as the test stock solution. Pipet a suitable volume of the test stock solution, dilute with sterile purified water so that each mL contains 30.0 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration test solution and low concentration test solution, respectively. Separately, weigh accurately about 30 mg (potency) of Josamycin RS, dissolve in 5 mL of methanol, add sterile purified water to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 5 °C, and use within 7 days. Pipet a suitable volume of the standard stock solution, add sterile purified water so that each mL contains 30.0 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively. Perform the test with these solutions as directed in I 8 under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Josamycin Tablets

Josamycin Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of josamycin ($C_{42}H_{69}NO_{15}$: 827.99).

Method of Preparation Prepare as directed under Tables, with Josamycin.

Identification To an amount of powdered Josamycin Tablets, equivalent to 10 mg (potency) of josamycin according to the labeled amount, add 100 mL of methanol, shake well, and centrifuge. To 5 mL of the clear supernatant liquid add methanol to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 229 and 233 nm.

Disintegration Test It meets the requirement.

Uniformity of Dosage Units Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Josamycin Tablets add 5 mL of water, and shake well to disintegrate. Add methanol, sonicate to disperse, add methanol to make V mL so that each mL contains about 2 mg (potency) of josamycin, and centriruge. Pipet 3 mL of the clear supernatant liquid, and add methanol to make exactly 100 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the test solution. Separately, weigh accurately about 50 mg (potency) of Josamycin RS, and dissolve in 5 mL of water and methanol to make exactly 25 mL. Pipet 3 mL of this solution, and add methanol to make exactly 100 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 231 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount [mg (potency)] of josamycin} = \text{Amount [mg (potency)] of Josamycin RS} \times \frac{A_T}{A_S} \times \frac{V}{25}$$

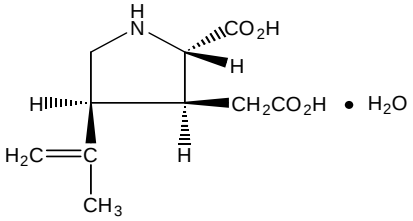
Loss on Drying Not more than 5.0 % (0.5 g, in vacuum, 60 °C, 3 hours).

Assay ***The Cylinder-plate method*** (1) Proceed as directed in the Assay under Josamycin.

(2) Weigh accurately and powder not less than 20 Josamycin Tablets. Weigh accurately a portion of the powder, equivalent to about 0.3 g (potency) of josamycin, add 50 mL of methanol, shake vigorously, and add water to make exactly 1000 mL. Pipet a suitable volume of this solution, add water so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration test solution and low concentration test solution, respectively.

Containers and Storage ***Containers***—Tight containers.

Kainic Acid Hydrate



$C_{10}H_{15}NO_4 \cdot H_2O$: 231.25

(2*S*,3*S*,4*S*)-3-(Carboxymethyl)-4-prop-1-en-2-ylpyrrolidine-2-carboxylic acid hydrate

Kainic Acid Hydrate, previously dried, contains not less than 99.0 % and not more than 101.0 % of kainic acid ($C_{10}H_{15}NO_4$: 213.23).

Description Kainic Acid Hydrate appears as white crystals or crystalline powder, is odorless and has an acid taste.

Kainic Acid Hydrate is sparingly soluble in water or in

warm water, very slightly soluble in ethanol (95) or in acetic acid (100) and practically insoluble in ether.
Kainic acid Hydrate dissolves in dilute hydrochloric acid or in sodium hydroxide TS.

Melting point—About 252 °C (with decomposition).

pH—The pH of a solution of Kainic Acid (1 in 100) is between 2.8 and 3.5.

Identification (1) To 5 mL of a solution of Kainic Acid Hydrate (1 in 5000), add 1 mL of ninhydrin TS and warm in a water-bath at a temperature between 60 °C and 70 °C for 5 minutes: a yellow color is produced.

(2) Dissolve 50 mg of Kainic Acid Hydrate in 5 mL of acetic acid (100) and add 0.5 mL of bromine TS: the color of bromine disappears immediately.

Specific Optical Rotation $[\alpha]_D^{20}$: -13 ~ -17° (0.5 g, water, 50 mL, 200 mm).

Purity (1) ***Clarity and color of solution***—Dissolve 0.10 g of Kainic Acid Hydrate in 10 mL of water: the solution is clear and colorless.

(2) ***Chloride***—Take 0.5 g of Kainic Acid Hydrate in a platinum crucible, dissolve in 5 mL of sodium carbonate TS and evaporate In a water-bath to dryness. Heat the crucible slowly at first and then ignite until the sample is almost incinerated. After cooling, add 12 mL of dilute nitric acid to the residue, dissolve by warming and filter. Wash the residue with 15 mL of water, combine the washings and the filtrate and add water to make 50 mL. Perform the test (not more than 0.021 %).

Control solution—Add 5 mL of sodium carbonate TS to 0.30 mL of 0.01 mol/L hydrochloric acid VS and proceed as directed above.

(3) ***Sulfate***—Dissolve 0.5 g of Kainic Acid Hydrate in 40 mL of water by warming. Cool, add 1 mL of dilute hydrochloric acid and water to make 50 mL and perform the test. Prepare the control solution with 0.30 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028 %).

(4) ***Ammonium***—Take 0.25 g of Kainic Acid Hydrate and perform the test. Prepare the control solution with 5.0 mL of standard ammonium solution (not more than 0.02 %).

(5) ***Heavy metals***—Proceed with 1.0 g of Kainic Acid Hydrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(6) ***Arsenic***—Dissolve 1.0 g of Kainic Acid Hydrate in 5 mL of dilute hydrochloric acid and perform the test (not more than 2 ppm).

(7) ***Related substances***—Dissolve 0.10 g of Kainic Acid Hydrate in 10 mL of water and use this solution as the test solution. Pipet 2.0 mL of this solution and add water to make exactly 100 mL. Pipet 1.0 mL of this solution, add water to make exactly 20 mL and use this solution as the standard solution. Perform the test as directed under the Thin-layer Chromatography with these solutions. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the supernatant liquid of a mixture of water, 1-butanol and acetic acid (100) (5 : 4 : 1) to a distance of about 10 cm and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate and dry the plate at 80 °C for 5 minutes: the spots other than the principal spot from the test solution are not more intense than the spot obtained form the standard solution.

Loss on Drying 6.5 ~ 8.5 % (1 g, 105 °C , 4 hours).

Residue on Ignition Not more than 0.1 % (0.5 g).

Assay Weigh accurately about 0.4 g of Kainic Acid Hydrate, previously dried and dissolve in 50 mL of warm water, cool and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 10 drops of bromothymol blue TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 21.323 mg of $C_{10}H_{15}NO_4$.

Containers and Storage ***Containers***—Tight containers.

Kallidinogenase

[*9001-01-8*]

Kallidinogenase is an enzyme obtained from healthy porcine pancreas, and has kinin-releasing activity based on vleavage of kininogen.
Kallidinogenase contains not less than 25 Kallidinogenase Units per mg.
Usually, Kallidinogenase is diluted with Lactose Hydrate or the like.
Kallidinogenase contains not less than 90.0 % and not more than 110.0 % of the labeled Units.

Description Kallidinogenase is a white to pale brown powder. Kallidinogenase is odorless or has a faint, characteristic odor.
Kallidinogenase is freely soluble in water, and practically insoluble in ethanol (95) or in ether.

pH—The pH of a solution of Kallidinogenase (1 in 300) is between 5.5 and 7.5.

Identification (1) Weigh accurately an appropriate amount of Kallidinogenase according to the labeled Units, and dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0 to prepare a solution containing 10 Kallidinogenase Units per mL. Pipet 5 mL of this solution, and add exactly 1 mL of trypsin inhibitor TS and 0.05 mol/L phosphate buffer solution, pH 7.0 to make

exactly 10 mL. Pipet 4 mL each of this solution into two separate test tubes, add exactly 1 mL each of aprotinin TS and 0.05 mol/L phosphate buffer solution, pH 7.0 separately to each test tube, allow them to stand at room temperature for 20 minutes, and use these solutions as the test solutions (1) and (2). Separately, pipet 1 mL of trypsininhibitor TS, and 0.05 mol/L phosphate buffer solution, pH 7.0 to mke exactly 10 mL. Pipet 4 mL each of this solution into two separate test tubes, add exactly 1 mL each of aprotinin TS and 0.05 mol/L phosphate buffer solution, pH 7.0 separately to each tube, allow them to stand at room temperature for 20 minutes, and use these solutions as the test solutions (3) and (4). Then, pipet 2.5 mL of substrate TS for Kallidinogenase assay (1), previously warmed at 30 ± 0.5 °C for 5 minutes, place in a 1-cm cell, add exactly 0.5 mL of the test solution (1) warmed at 30 ± 0.5 °C for 5 minutes, and start simultaneously a chronograph. Perform the test at 30 ± 0.5 °C as directed under Ultraviolet-visible Spectrophotometry using water as the blank, and determine the absorbances at 405 nm, $A_{1\text{-}2}$and $A_{1\text{-}6}$, of this solution, after having allowed it to stand for exactly 2 and 6 minutes. Perform the same test with the test solutions (2), (3) and (4), and determine the absorbances, $A_{2\text{-}2}$, $A_{2\text{-}6}$, $A_{3\text{-}2}$, $A_{3\text{-}6}$, $A_{4\text{-}2}$ and $A_{4\text{-}6}$, of these solutions. Calculate I by using the following equation: the value of I does not exceed 0.2.

$$\mathrm{I} = \frac{(A_{1-6} - A_{1-2}) - (A_{3-6} - A_{3-2})}{(A_{2-6} - A_{2-2}) - (A_{4-6} - A_{4-2})}$$

(2) Pipet 2.9 mL of substrate TS for Kallidinogenase assay (2), previously warmed at 30 ± 0.5 °C for 5 minutes, place in a 1-cm cell, add exactly 0.1 mL of the test solution obtained in the Assay, and start simultaneously a chromatograph. Perform the test at 30 ± 0.5 °C as directed under Ultraviolet-visible Spectrophotometry, and determine the change of the absorbance at 253 nm for to inhibitor TS, and add 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL. Add exactly 0.1 mL of this solution to exactly 2.9 mL of substrate TS for Kallidinogenase assay (2), previously warmed at 30 ± 0.5 °C for 5 minutes, and use this solution as the blank. If the rate of change in the absorbance remains constant, determine the change of absorbance per 1 minute, A, and calculate R by using the following equation: the value of R is between 0.12 and 0.16.

$$R = \frac{A}{0.0383} \times \frac{1}{a - b}$$

a: Amount (mg) of kallidinogenase in 1 mL of the test solution.

b: Amount (unit) of kallidinogenase in 1 mg of Kallidinogenase obtained in the Assay.

Specific Activity Perform the test with Kallidinogenase as directed under Nitrogen Determination to determine the nitrogen content, convert 1 mg of nitrogen (N:14.01) into 6.25 mg of protein, and calculate the specific activity using the amount (Units) of kallidinogenase obtained in the Assay: it is not less than 100 Kallidinogenase Units per 1 mg of protein.

Purity (1) ***Fat***—To 1.0 g of Kallidinogenase add 20 mL of ether, extract with occasional shaking for 30 minutes, and filter. Wash the residue with 10 mL of ether, combine the washing with the filtrate, evaporate the ether, and dry the residue at 105 °C for 2 hours: the mass of the residue is not more than 1 mg.

(2) ***Kininase***—(i) Bradykinin solution: Weigh an appropriate amount of bradykinin, and dissolve in gelatin-phosphate buffer solution, pH 7.4 to prepare a solution containing 0.200 μg of bradykinin per mL.

(ii) Kallidinogenase solution: Weigh accurately a suitable amount of Kallidinogenase according to the labeled unit, dissolve in gelatin-phosphate buffer solution, pH 7.4 to make a solution containing 1 unit of Kallidinogenase per mL.

(iii) Test solution: Pipet 0.5 mL of bradykinin solution, warm at 30 ± 0.5 °C for 5 minutes, then add exactly 0.5 mL of Kallidinogenase solution previously warmed at 30 ± 0.5 °C for 5 minutes, and mix immediately. After allow this solution to stand at 30 ± 0.5 °C for exactly 150 seconds, add exactly 0.2 mL of a solution of trichloroacetic acid (1 in 5), and shake. Boil for 3 minutes, then cool in ice immediately, centrifuge, and allow to stand at a room temperature for 15 minutes. Pipet 0.5 mL of the supernatant liquid, add exactly 0.5 mL of gelatin-tris buffer solution, pH 8.0, and mix. Pipet 0.1 mL of this solution, add exactly 0.9 mL of trichloroacetic acid –gelatin-tris buffer solution, and mix. Pipet 0.2 mL of this solution, add exactly 0.6 mL of trichloroacetic acid- gelatin-tris buffer solution, shake, and use this solution as the test solution.

(iv) Control solution: Proceed with 0.5 mL of gelatin-phosphate buffer solution, pH 7.4 as described in (iii), and use the solution so obtained as the control solution.

(v) Procedure: Add 0.1 mL of anti-bradykinin antibody TS to anti-rabbit antibody-coated wells of a 96-well microplate, shake, and allow to stand at a constant temperature of about 25 °C for 1 hour. Remove the anti-bradykinin antibody TS, add 0.3 mL of phosphate buffer solution for microplate washing to the wells, then remove. Repeat this procedure 3 times, take off the washings thoroughly, then add 100 μL each of the test solution and the control solution, and 50 μL of gelatin-phosphate buffer solution, pH 7.0, shake, and allow to stand at a constant temperature of about 25 °C for 1 hour. Then add 50 μL of peroxidase-labeled bradykinin TS, shake, and allow to stand in a cold place for a night. Take off the solution, add 0.3 mL of phosphate buffer solution for microplate washing, and remove. Repeat this procedure more 4 times, take off the washings thoroughly, add 100 μL of substrate solution for peroxidase determination, and allow to stand as

a constant temperature of about 25 °C for exactly 30 minutes while protecting from light. Then add 100 μL of diluted surfuric acid (23 in 500), shake, and determine the absorbance at 490 ~ 492 nm. Separately, dissolve a suitable amount of bradykinin in gelatin-phosphate buffer solution, pH 7.0 to make solution containing exactly 100 ng, 25 ng, 6.25 ng, 1.56 ng, 0.39 ng and 0.098 ng of bradykinin per mL, and use these solutions as the standard solution (1), the standard solution (2), the standard solution (3), the standard solution (4), the standard solution (5), the standard solution (6), respectively. Use 1 mL of gelatin-phosphate buffer solution, pH 7.0 as the standard solution (7). To each of the well add 50 μL each of the standard solutions and 100 μL of trichloroacetic acid-gelatin-tris buffer solution, and proceed in the same manner as for the test solution and for the control solution. Prepare the standard curve from the amounts of bradykinin in the standard solutions and their absorbances, and determine the amount of bradykinin, B_T (pg) and B_S (pg), of the test solution and the control solution. The absorbance is usually determined by using a spectrophotometer for microplate. Since the wells are used as the cell for absorbance determination, take care for dirt and scratch of the well. Light pass length of the well is changeable by the amount of the liquid, exact addition of the liquid is necessary.

(vi) Judgement: The value R calculated by the following equation is not less than 0.8.

$$R = (B_T / B_S)$$

(3) ***Trypsin-like substances***—Pipet 4 mL of the test stock solution prepared for the Assay, add exactly 1 mL of trypsin inhibitor TS and 0.05 mol/L phosphate buffer solution , pH 7.0 to make exactly 10 mL, and use this solution as the test solution. Pipet 2.5 mL of substrate TS for kallidinogenase assay (1), previously warmed at 30 ± 0.5 °C for 5 minutes, place in a 1-cm cell, add exactly 0.5 mL of the test solution, warmed at 30 ± 0.5 °C for 5 minutes, and start simultaneously a chronograph. Perform the test at 30 ± 0.5 °C as directed under Ultraviolet-visible Spectrophotometry using water as the blank, and determine the absorbances at 405 nm, A_2 and A_6, of this solution after having allowed it to stand for exactly 2 and 6 minutes. Seperately, pipet 4 mL of the test stock solution prepared for the Assay, add 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL, and use this solution as the control solution. Perform the same test with the control solution, and determine the absorbances, A'_2 and A'_{26}. Calculate T by using the following equation: the value of T does not exceed 0.05.

$$T = \{(A'_6 - A'_2) - (A_6 - A_2) / (A'_6 - A'_2)\}$$

(4) ***Protease***—Weigh accurately an appropriate amount of kallidinogenase according to the labeled Units, dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0 to prepare a solution containing 1 Kallidinogenase Unit per mL, and use this solution as the test solution. Pipet 1 mL of the test solution, place in a test tube, and allow to stand at 35 ± 0.5 °C for 5 minutes. Then, pipet 5 mL of substrate TS for kallidinogenase assay (3), previously warmed to 35 ± 0.5 °C add quickly to the test solution in the test tube, and allow to stand at 35 ± 0.5 °C for exactly 20 minutes. Then add exactly 5 mL of trichloroacetic acid TS, shake well, allow to stand at room temperature for 1 hour, and filter through a membrane filter (5 μm in pore size). Discard the first 3 mL of the filtrate, and determine the absorbance, A, of the subsequent filtrate at 280 nm within 2 hours as directed under Ultraviolet-visible Spetrophotometry, using water as the blank. Separately, pipet 1 mL of the test solution, add exactly 5 mL of trichloroacetic acid TS, shake well, and add exactly 5 mL of the substrate TS for kallidinogenase assay (3). Proceed in the same manner as described for the test solution, and determine the absorbance, A_0, of this solution. Calculate the value of (A - A_0): it is not more than 0.2.

Loss on Drying Not more than 2.0 % (0.5g, in vacuum, P_2O_5, 4 hours).

Residue on Ignition Not more than 3.0 % (0.5g, 650~750 °C).

Kinin-Releasing Activity (i) Kallidinogenase solution: Weigh accurately a suitable amount of Kallidinogenase, according to the labeled unit, dissolve in 0.02 mol/L phosphate buffer solution, pH 8.0 to make a solution containing 0.1 unit of Kallidinogenase per mL. Perform this procedure by using glassware.

(ii) Test solution: Pipet 0.5 mL of kininogen TS, warm at 30 ± 0.5 °C for 5 minutes, then add exactly 0.5 mL of Kallidinogenase solution previously warmed at 30 ± 0.5 °C for 5 minutes, and mix immediately. After allow this solution to stand at 30 ± 0.5 °C for exactly 2 minutes, add exactly 0.2 mL of a solution of trichloroacetic acid (1 in 5), and shake. Boil for 3 minutes, then cool in ice immediately, centrifuge, and allow to stand at a temperature for 15 minutes. Pipet 0.5 mL of the supernant liquid, addd exactly 0.5 mL of gelatin-tris buffer solution, pH 8.0, and shake. Pipet 0.1 mL of this solution, add exactly 1.9 mL of trichloroacetic acid-gelatin-tris buffer solution, shake, and use this solution as the test solution.

(iii) Procedure: Perform the test with the test solution as directed in the Purity (2), and determine the amount, B (pg), of kinin per well. The kinin-releasing activity per 1 unit of Kallidinogenase calculated by the following equation is not less than 500 ng bradykinin equivalent/min/unit.

Kinin-releasing activity (ng bradykinin equivalent/min/unit) per 1 unit of Kallidinogenase
$= B \times 4.8$

Assay Weigh accurately an appropriate amount of Kallidinogenase according to the labeled units, dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0 to prepare a solution containing about 10 Kallidinogenase Units per mL, and use this solution as the test stock solution. Pipet 4 mL of the test stock solution, add exactly 1 mL of trypsin inhibitor TS and 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL, and use this solution as the test solution. Pipet 2.5 mL of substrate TS for Kallidinogenase assay (1), previously warmed at 30 ± 0.5 °C for 5 minutes, place in a 1-cm cell, add exactly 0.5 mL of the test solution, warmed at 30 ± 0.5 °C for 5 minutes, and start simultaneously a chronograph. Perform the test at 30 ± 0.5 °C as directed under Ultraviolet-visible Spetrophotometry using water as the blank, and determine the absorbances at 405 nm, A_{T2} and A_{T6}, of this solution after allowing to stand for exactly 2 and 6 minutes. Separately, dissolve Kallidinogenase reference standard in 0.05 mol/L phosphate buffer solution, pH 7.0 to make a solution so that each mL contains exactly 10 Units, and use this solution as the standard stock solution. Pipet 4 mL of the stock solution, add exactly 1 mL of trypsin inhibitor TS and 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL, and use this solution as the standard solution. Take exactly 0.5 mL of the standard solution, perform the test in the same manner as described for the test solution, and determine the absorbances, A_{S2} and A_{S6}, of the solution after allowing to stand for exactly 2 and 6 minutes. Separately, take exactly 1 mL of the trypsin inhibitor TS, and add 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL. Pipet 0.5 mL of this solution, perform the test in the same manner as described for the test solution, and determine the absorbances, A_{02} and A_{06}, of the solution after allowing to stand for exactly 2 and 6 minutes.

$$\text{Units per 1 mg of Kallidinogenase} = \frac{(A_{T6} - A_{T2}) - (A_{06} - A_{02})}{(A_{S6} - A_{S2}) - (A_{06} - A_{02})} \times \frac{W_S}{a} \times \frac{1}{b}$$

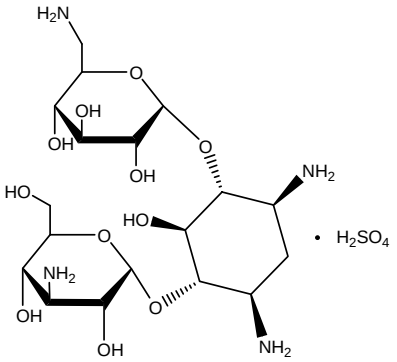
W_S: Amount (Units) of Kallidinogenase Reference Standard

a: Volume (mL) of the standard stock solution

b: Amount (mg) of Kallidinogenase in 1 mL of the test stock solution

Containers and Storage ***Containers***—Tight containers.

Kanamycin Monosulfate



$C_{18}H_{36}N_4O_{11}\cdot H_2SO_4$: 582.58

(2*R*,3*S*,4*S*,5*R*,6*R*)-2-(Aminomethyl)-6-{[(1*R*,2*R*,3*S*,4*R*,6*S*)-4,6-diamino-3-{[(2*S*,3*R*,4*S*,5*S*,6*R*)-4-amino-3,5-dihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}-2-hydroxycyclohexyl]oxy}oxane-3,4,5-triol monosulfate [*25389-94-0*]

Kanamycin Monosulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of *Streptomyces kanamyceticus*.
Kanamycin Monosulfate contains not less than 750 μg (potency) per mg of kanamycin ($C_{18}H_{36}N_4O_{11}$: 484.50), calculated on the dried basis.

Description Kanamycin Monosulfate appears as white crystalline powder
Kanamycin Monosulfate is freely soluble in water, and practically insoluble in ethanol (99.5).

Identification (1) To 50 mg of Kanamycin Monosulfate add 3 mL of water and dissolve, and add 6 mL of anthrone TS: a blue-purple color develops.

(2) Dissolve separately 20 mg each of Kanamycin Monosulfate and Kanamycin Monosulfate RS in 1 mL of water, and use these solutions as the test solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the clear supernatant liquid of a mixture of chloroform, ammonia solution (28), and methanol (2 : 1 : 1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2 % ninhydrin-water saturated 1-butanol TS on the plate, and heat at 100 °C for 10 minutes: the principal spot obtained from the test solution and the spot from the standard solution are purple-brown in color and have the same R_f value.

(3) To a solution of Kanamycin Monohydrate (1 in 5) add one drop of barium chloride TS: a white precipitate is produced.

Specific Optical Rotation $[\alpha]_D^{20}$: +112 ~ +123° (0.2 g calculated on the dried basis, water, 20 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.1 g of Kanamycin Monosulfate in 10 mL of water is between 6.5 and 8.5.

Purity (1) ***Sulfate content***—Weigh accurately 0.25 g of Kanamycin Monosulfate, dissolve in 100 mL of water, and adjust the pH to 11.0 with ammonia solution (28). To this solution add exactly 10 mL of 0.1 mol/L barium chloride VS, and titrate with 0.1 mol/L disodium ethylenediaminetetraacetate VS (indicator: phthalein purple, 0.5 mg). When the color of the solution begins to change add 50 mL of ethanol (99.5) and titrate until the blue-purple color of the solution disappears. Perform a blank determination. The amount of sulfate (SO_4^{2-}) is not less than 15.0 % and not more than 17.0 %, calculated on the dried basis.

Each mL of 0.1 mol/L barium chloride VS
= 9.606 mg of sulfate (SO_4^{2-})

(2) ***Heavy metals***—Proceed with 2.0 g of Kanamycin Monosulfate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Proceed with 2.0 g of Kanamycin Monosulfate according to Method 4, and perform the test (not more than 1 ppm).

(4) ***Related substances***—Weigh accurately 0.30 g of Kanamycin Monosulfate, dissolve in water to make exactly 10 mL, and use this solution as the test solution. Separately, weigh accurately 45 mg of Kanamycin Monosulfate RS, dissolve in water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 1 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (3 in 40) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in 1-butanol (1 in 100) on the plate, and heat at 110 °C for 10 minutes: the spot other than the principal spot from the test solution is not more intense than the spot from the standard solution.

Loss on Drying Not more than 4.0 % (5 g, reduced pressure not exceeding 0.67 kPa, 60 °C, 3 hours).

Residue on Ignition Not more than 0.5 % (1 g).

Sterility Test It meets the requirement, when Kanamycin Monosulfate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.50 EU/mg of kanamycin, when Kanamycin Monosulfate is used in a sterile preparation.

Abnormal Toxicity Dissolve 2 mg of Kanamycin Monosulfate in 0.5 mL of water for injection, and inject intravenously for 15 to 30 seconds to each of 5 healthy mice weighing 17 to 24 g. Use animals in which no abnormalities are observed for not less than 5 days prior to the test. No animals die during the 24 hour post-dosage observation. If 1 animal dies, repeat the test with 5 animals: no animals die during the 24 hour observation.

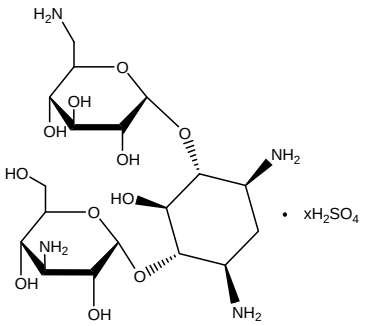
Assay ***The Cylinder-plate method*** (1) Agar media for seed and base layer- Use the medium in I 2 1) (1) ① under Microbial Assay for Antibiotics.

(2) Test organism- *Bacillus subtilis* ATCC 6633.

(3) Weigh accurately about 20 mg (potency) of Kanamycin Monosulfate, and dissolve in water to make exactly 50 mL. Pipet a suitable volume of this solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) so that each mL contains 20.0 μg (potency) and 5.0 μg (potency), and use these solutions as the high concentration test solution and low concentration test solution, respectively. Separately, weigh accurately about 20 mg (potency) of Kanamycin Monohydrate RS, previously dried, dissolve in diluted pH 6.0 phosphate buffer solution (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution between 5 and 15 °C, and use within 30 days. Pipet a suitable volume of the standard stock solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) so that each mL contains 20.0 μg and 5.0 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively. Perform the test with these solutions as directed in I 8 under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Well-closed containers.

Kanamycin Sulfate



$C_{18}H_{36}N_4O_{11}\cdot xH_2SO_4$

(2*R*,3*S*,4*S*,5*R*,6*R*)-2-(Aminomethyl)-6-{[(1*R*,2*R*,3*S*,4*R*,6*S*)-4,6-diamino-3-{[(2*S*,3*R*,4*S*,5*S*,6*R*)-4-amino-3,5-dihydroxy-6-(hydroxymethyl)oxan-2-

yl]oxy}-2-hydroxycyclohexyl]oxy}oxane-3,4,5-triol sulfate [*25389-94-0*]

Kanamycin Sulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of *Streptomyces kanamyceticus*.
Kanamycin Sulfate contains not less than 690 μg (potency) and not more than 740 μg (potency) per mg of kanamycin ($C_{18}H_{36}N_4O_{11}$: 484.50), calculated on the dried basis.

Description Kanamycin Sulfate appears as white to yellowish white powder.
Kanamycin Sulfate is very soluble in water, and practically insoluble in ethanol (99.5).

Identification (1) Dissolve separately 20 mg each of Kanamycin Sulfate and Kanamycin Monosulfate RS in 1 mL of water, and use these solutions as the test solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, ammonia solution (28), and methanol (2 : 1 : 1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2 % ninhydrin-water saturated 1-butanol TS on the plate, and heat at 100 °C for 10 minutes: the principal spot obtained from the test solution and the spot from the standard solution are purple-brown in color and have the same R_f value.

(2) A solution of Kanamycin Sulfate (1 in 10) responds to the Qualitative Tests (1) for sulfate.

Specific Optical Rotation $[\alpha]_D^{20}$: +103 ~ +115° (0.5 g calculated on the dried basis, water, 50 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g of Kanamycin Sulfate in 20 mL of water is between 6.0 and 7.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.5 g of Kanamycin Sulfate in 5 mL of water: the solution is clear and colorless to pale yellow.

(2) ***Heavy metals***—Proceed with 1.0 g of Kanamycin Sulfate according to Method 4, and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

(3) ***Arsenic***—Proceed with 2.0 g of Kanamycin Sulfate according to Method 3, and perform the test (not more than 1 ppm).

(4) ***Related substances***—Weigh accurately 0.30 g of Kanamycin Sulfate, dissolve in water to make exactly 10 mL, and use this solution as the test solution. Separately, weigh accurately 9.0 mg of Kanamycin Monohydrate RS, dissolve in water to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 1 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (3 in 40) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in 1-butanol (1 in 100) on the plate, and heat at 110 °C for 10 minutes: the spot other than the principal spot obtained from the test solution is not more intense than the spot from the standard solution.

Loss on Drying Not more than 5.0 % (0.5 g, reduced pressure not exceeding 0.67 kPa, 60 °C, 3 hours).

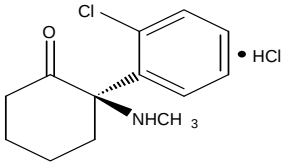
Sterility Test It meets the requirement, when Kanamycin Sulfate is used in a sterile preparation.

Pyrogen Test It meets the requirement, when Kanamycin Sulfate is used in a sterile preparation,. Weigh an appropriate amount of Kanamycin Sulfate, dissolve in water, make the solution so that each mL contains 10.0 mg, and use the solution as the test solution. The amount of injection is 1.0 mL of the test solution per kg of body weight of rabbit.

Assay Perform the test according to Assay in Kanamycin Monosulfate. Weigh accurately about 20 mg (potency) of Kanamycin Suflate, and dissolve in water to make exactly 50 mL.

Containers and Storage ***Containers***—Tight containers.

Ketamine Hydrochloride



and enantiomer

$C_{13}H_{16}ClNO \cdot HCl$: 274.19

2-(2-Chlorophenyl)-2-(methylamino)cyclohexan-1-one hydrochloride [*1867-66-9*]

Ketamine Hydrochloride, when dried, contains not less than 99.0 % and not more than 101.0 % of ketamine hydrochloride ($C_{13}H_{16}ClNO \cdot HCl$).

Description Ketamine Hydrochloride appears as white crystals or crystalline powder.
Ketamine Hydrochloride is very soluble in formic acid, freely soluble in water or in methanol, sparingly soluble in ethanol (95) or in acetic acid (100) and practically insoluble in acetic anhydride or in ether.
A solution of Ketamine Hydrochloride (1 in 10) shows no optical rotation.

Melting point—258 °C (with decomposition).

Identification (1) Determine the absorption spectra of solutions of Ketamine Hydrochloride and Ketamine Hydrochloride RS in 0.1 mol/L hydrochloric acid TS (1 in 3000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same waveleng.

(2) Determine the infrared spectra of Ketamine Hydrochloride and Ketamine Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Ketamine Hydrochloride (1 in 10) responds to the Qualitative Tests (2) for chloride.

pH Dissolve 1.0 g of Ketamine Hydrochloride in 10 mL of freshly boiled and cool water: the pH of this solution is between 3.5 and 4.5.

Absorbance $E_{1\,cm}^{1\%}$ (269 nm): 22.0 ~ 24.5 (after drying, 30 mg, 0.1 mol/L hydrochloric acid TS, 100 mL).

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Ketamine Hydrochloride in 5 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Ketamine Hydrochloride according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Ketamine Hydrochloride, according to Method 1 and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 0.5 g of Ketamine Hydrochloride in 10 mL of methanol and use this solution as the test solution. Pipet 1.0 mL of the test solution, add methanol to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 2 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and isopropylamine (49 : 1) to a distance of about 10 cm and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate, dry the plate and then spray evenly hydrogen peroxide TS: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

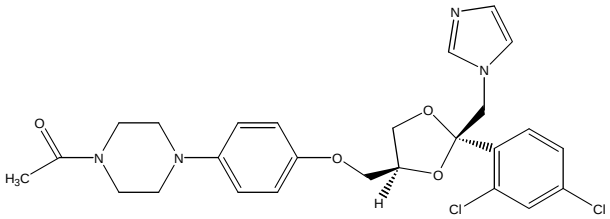
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.5 g of Ketamine Hydrochloride, previously dried, dissolve in 1 mL of formic acid, add 70 mL of a mixture of acetic anhydride and acetic acid (100) (6 : 1) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 27.419 mg of $C_{13}H_{16}ClNO \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Ketoconazole



and enantiomer

$C_{26}H_{28}Cl_2N_4O_4$: 531.43

1-[4-(4-{[2-(2,4-Dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy}phenyl)piperazin-1-yl]ethan-1-one
[*65277-42-1*]

Ketoconazole contains not less than 98.0 % and not more than 102.0 % of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$), calculated on the dried basis.

Description Ketoconazole appears as white powder. Ketoconazole is soluble in methanol, sparingly soluble in ethanol (95), and practically insoluble in water.

Identification Determine the infrared spectra of Ketoconazole and Ketoconazole RS, as directed in the potassium bromide disk method under Infrared spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 148 ~ 152 °C.

Specific Optical Rotation $[\alpha]_D^{20}$: -1 ~ +1° (0.4 g, methanol, 10 mL, 100 mm).

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Ketoconazole according to Method 2 under Heavy Metals Limit Test, and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Dissolve 0.10 g of Ketoconazole in 10 mL of methanol, and use this solution as the test solution. Pipet 5 mL of this solution, and add methanol to make exactly 100 mL. Pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solu-

tion as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method: the area of the peak other than ketoconazole from the test solution is not more than 2/5 times the peak area of ketoconazole from the standard solution. The total area of the peaks other than ketoconazole from the test solution is not larger than the peak area of ketoconazole from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 10 cm in length, packed with octadecylsilaznied silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Control the step or concentration gradient by mixing mobile phases A and B as directed in the following table.

Mobile phase A: Acetonitrile

Mobile phase B: A solution of tetrabutylammonium hydrogen sulfate in ammonium (17 in 5000)

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-10	5→50	95→50
10-15	50	50

Flow rate: 2.0 mL/minute

System suitability

Test for required detectability: Pipet 2 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of ketoconazole obtained from 10 μL of this solution is equivalent to 7 to 13 % of that of ketoconazole from the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of ketoconazole are not less than 40000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of ketoconazole is not more than 2.5 %.

Time span of measurement: For 15 minutes after injection, beginning after the solvent peak.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, 80 °C , 4 hours).

Residue on Ignition Not more than 0.1 % (2 g).

Assay Weigh accurately about 0.2 g of Ketoconazole, dissolve in 40 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 26.572 mg of $C_{26}H_{28}Cl_2N_4O_4$

Containers and Storage ***Containers***—Tight containers.

Ketoconazole Tablets

Ketoconazole Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$: 531.43).

Method of Preparation Prepare as directed under Tablets, with Ketoconazole.

Identification Weigh accurately and powder a quantity of Ketoconazole Tablets equivalent to about 50 mg of Ketoconazole, add 50 mL of chloroform, shake for 2 minutes, filter, and use the filtrate as the test solution. Separately, dissolve a suitable quantity of Ketoconazole RS in chloroform to make a solution containing 1 mg per mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of *n*-hexane, ethyl acetate, methanol, water and acetic acid (100) (42 : 40 : 15 : 2 : 1) to a distance of about 15 cm, and air-dry. And examine the plate under ultraviolet light (main wavelength: 254 nm): the R_f value of the principal spot obtained from the test solution corresponds to that obtained from the standard solution.

Dissolution Test Perform the test with 1 tablet of Ketoconazole Tablets at 50 revolutions per minute according to Method 2 under Dissolution Test, using 900 mL of 0.1 mol/L hydrochloric acid TS as the dissolution solution. Take not less than 20 mL of the dissolved solution 30 minutes after the start of the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution solution to make exactly V' mL, and use this solution as the test solution. Separately, weigh accurately a suitable amount of ketoconazole RS, dissolve in the dissolution solution to make the same concentration as the test solution, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 270 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry, using the dissolution solution as the blank. The dissolution rate of Ketoconazole Tablets in 30 minutes is not less than 80 % (Q).

Dissolution rate (%) with respect to the labeled amount of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$)

$$= C_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 90000$$

C_S: Concentration (mg/mL) of the standard solution

C: Labeled amount (mg) of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$) in 1 tablet

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Ketoconazole Tablets. Weigh accurately a portion of the powder, equivalent to about 0.2 g of Ketoconazole, transfer to a glass-stoppered centrifuge tube and add exactly 50 mL of a mixture of methanol and dichloromethane (1 : 1). Shake for 30 minutes with a shaker and centrifuge. Pipet 5 mL of the supernatant liquid, add 5 mL of the internal standard solution and add a mixture of methanol and dichloromethane (1 : 1) to make exactly 50 mL. Use this solution as the test solution. Separately, weigh accurately about 20 mg of Ketoconazole RS, add 5 mL of the internal standard solution and add a mixture of methanol and dichloromethane (1 : 1) to make exactly 50 mL. Use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of ketoconazole to that of the internal standard for the test solution and the standard solution, respectively.

Amount (mg) of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$)

$$= \text{Amount (mg) of Ketoconazole RS} \times \times \frac{Q_T}{Q_S} \times 10$$

Internal standard solution—Weigh a suitable quantity of Terconazole RS and dissolve in a mixture of methanol and dichloromethane (1 : 1) to make a solution containing 5 mg per mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column, about 3.9 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm to 10 μm in particle diameter).

Mobile phase: A mixture of diisopropylamine in methanol (1 in 500) and ammonium acetate in water (1 in 200) (7 : 3).

Flow rate: 3 mL/minute.

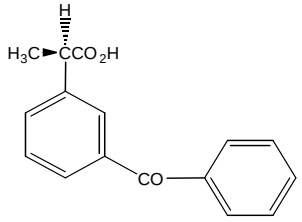
System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, ketoconazole and the internal standard are eluted in this order with a resolution between their peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ketoconazole to that of the internal standard is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Ketoprofen



and enantiomer

$C_{16}H_{14}O_3$: 254.28

2-(3-Benzoylphenyl)propanoic acid [*22071-15-4*]

Ketoprofen, when dried, contains not less than 99.0 % and not more than 100.5 % of ketoprofen ($C_{16}H_{14}O_3$).

Description Ketoprofen is a white, crystalline powder.

Ketoprofen is very soluble in methanol, freely soluble in ethanol (95) or in acetone and practically insoluble in water.

A solution of Ketoprofen in ethanol (99.5) (1 in 100) shows no optical rotation.

Ketoprofen is colored to pale yellow by light.

Identification (1) Determine the absorption spectra of solutions of Ketoprofen and Ketoprofen RS in methanol (1 in 200000) as directed under Ultraviolet-visible Spectrophotomtry: both spectra exhibit similar intensities of absorption at the same wavelength.

(2) Determine the infrared spectra of Ketoprofen and Ketoprofen RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the wavenumbers.

Melting Point 94 ~ 97 °C.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Ketoprofen in 10 mL of acetone: the solution is clear and has no more color than the following control solution.

Control solution—To a mixture of 0.6 mL of cobalt (II) chloride hexahydrate colorimetric stock solution

and 2.4 mL of iron (III) chloride hexahydrate colorimetric stock solution add the diluted hydrochloric acid (1 in 10) to make 10 mL. To 5.0 mL of this solution add diluted hydrochloric acid(1 in 10) to make 100 mL.

(2) ***Heavy metals***—Proceed with about 2.0 g of Ketoprofen according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***— Perform this procedure with a minimum of exposure to light, using light-resistant vessels. Dissolve 20 mg of Ketoprofen in 20 mL of the mobile phase and use this solution as the test solution. Pipet 1 mL of the test solution and add the mobile phase to make exactly 50 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 mL and use this solution as the standard solution. Perform the test with exactly 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area by the automatic integration method: the areas of the peaks from the test solution, having the relative retention time of about 1.5 and about 0.3 with respect to ketoprofen are not greater than 4.5 times and 2 times the peak area of ketoprofen from the standard solution, respectively. The area of the peak other than the peaks with the relative retention times of about 1.5 and about 0.3 and other than ketoprofen from the test solution is not larger than the peak area of ketoprofen from the standar solution, and the total area of these peaks is not larger than 2 times the peak area of ketoprofen from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometrer (wavelength : 233 nm)

Column: A stainlenn steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 68.0 g of potassium dihydro phosphate in water to make 1000mL, and adjust the pH to 3.5 with phosphoric acid, To 20 mL of this solution add 430 mL of acetonitrile and 550 mL of water.

Flow rate: Adjust the flow rate so that the retention time of ketoprofen is about 7 minutes.

System suitability

Test for required detectability: To exactly 1 mL of the standard solution, add the mobile phase to make exactly 10 mL. Confirm that the peak area of ketoprofen obtained with 20 μL of this solution is equivalent to 9 to 11 % of that with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operation conditions, the number of the theoretical plates and symmetry factor the peak of ketoprofen is not less than 8000 and not more than 1.5 respectively.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ketoprofen is not more than 2.0 %.

Time span of measurement: About 7 times as long as the retention time of ketoprofen.

Loss on Drying Not more than 0.5 % (0.5 g, in vacuum, 60 °C, 24 hours).

Residue on Ignition Not more than 0.1 % (1 g).

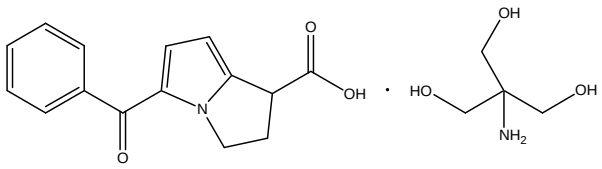
Assay Weigh accurately about 0.3 g of Ketoprofen, previously dried, dissolve in 25 mL of ethanol (95), add 25 mL of water and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 25.428 mg of $C_{l6}H_{14}O_3$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Ketorolac Tromethamine



$C_{15}H_{13}NO_3 \cdot C_4H_{11}NO_3$: 376.40

2-Amino-2-(hydroxymethyl)propane-1,3-diol; 5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylic acid [*74103-07-4*]

Ketorolac Tromethamine contains not less than 98.5 % and not more than 101.5 % of ketorolac tromethamine ($C_{15}H_{13}NO_3 \cdot C_4H_{11}NO_3$), calculated on the dried basis.

Description Ketorolac Tromethamine is a white, crystalline powder.

Ketorolac Tromethamine is freely soluble in water or in methanol, sparingly soluble in ethanol (95), in ethanol (99.5) or in tetrahydrofuran, and practically insoluble in acetonitrile, in acetone, in dichloromethane, in toluene, in ethyl acetate, in 1,4-dioxane, in hexane, or in butanol.

Melting point—165 ~ 170 °C (with decomposition).

Identification (1) Determine the absorption spectra

of solutions of Ketorolac Tromethamine and Ketorolac Tromethamine RS in methanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelength.

(2) Determine the infrared spectra of Ketorolac Tromethamine and Ketorolac Tromethamine RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumber.

(3) Dissolve each 50 mg each of Ketorolac Tromethamine and Ketorolac Tromethamine RS in a mixture of dichloromethane and methanol (2:1) to make 10 mL, and use these solutions as the test solution and the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spots each 40 μL of the test solution and the standard solution on a plate of silica gel for the thinlayer chromatography. Develop the plate with a mixture of dichloromethane, acetone and acetic acid (100) (95 : 5 : 2) to a distance of about 15 cm, and air-dry the plate. Sprlay evenly a solution of ninhydrin in ethanol (95) (0.3 in 10) on the plate and heat the plate at about 150 °C for 2 to 5 minutes: yellow spots with pink to purple borders is shown.

pH Dissolve 1 g of Ketorolac Tromethamine in 100 mL of water: the pH of this solution is between 5.7 and 6.7.

Purity (1) ***Heavy metals***— Proceed with 1.0 g of Ketorolac Tromethamine according to the method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***— Perform the test with 10 μL of the test solution as directed under Liquid Chromatography according to following operating conditions. Determine each peak area by the automatic integration method, and calculate the amount of each related substances: the ketorolac 1-keto analog or the ketorolac 1-hydroxy analog is not more than 0.1 % and any other related substance is not more than 0.5 % and the sum of all related substances is not more than 1.0 %.

$$\text{Amount (\%) of related substances} = 100 \times rf_1 \times \frac{A_i}{A_S}$$

rf_1: Response factor of each individual related substance peak relative to that of ketorolac; the rf_1 values are:

0.52 for the ketorolac 1- to analog, 0.67 for the ketorolac 1-hydroxy analog,

2.2 for the related substance peak having a retention time of 0.54 relative to that of ketorolac, and

0.91 for the related substance peak at a relative retention time of 0.66.

A_i: Peak area of each related substance.

A_S: Sum of all the peak areas of the related substance peaks and the principal ketorolac peak.

Operating conditions

Test solution, standard solution, resolution solution, detector, column column temperature, mobile phase, flow rate, and system suitability: Proceed as directed the in operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of ketorolac.

Loss on Drying not more than 0.5 % (1 g, 60 °C, 3 hours).

Residue on Ignition not more than 0.1 % (1 g).

Assay Weigh accurately about 20 mg each of Ketorolac Tromethamine and Ketorolac Tromethamine RS, dissolve in a mixture of water and tetrahydrofuran (70 : 30) to make exactly 50 mL, and use these solutions as the test solution and the standard solution. The test solution and the standard solution are protected from light. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Calculate the area of the principal peak of each solutions, A_T and A_S, by the automatic integration method.

$$\text{Amount(mg) of ketorolac tromethamine } (C_{15}H_{13}NO_3 \cdot C_4H_{11}NO_3)$$
$$= \text{Amount (mg) of Ketorolac Tromethamine RS} \times \frac{A_T}{A_S}$$

Operating conditions

Dtector: An ultraviolet absortion photometer (wavelength: 313 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: A mixture of ammonium phosphate buffer and tetrahydrofuran (70 : 30).

Flow rate**:** 1.5 mL/minute.

System suitability

System performance: In a 250-mL separator, mix 100 mL of water, 100 mL of dichloromethane, 30 mg of Ketorolac Tromethamine RS and 1 mL of 1 mol/L hydrochloric acid, shake, and allow the layers to separate. Transfer the lower dichloromethane to a flask, and discard the upper layer. Expose the dichloromethane solution to direct sunlight 10 to 15 minutes. Transfer 1.0 mL of this solution to a vial, evaporate in a current of air or in a stream of nitrogen to dryness, add 1.0 mL of a mixture of water and tetrahydrofuran (70 : 30), and dissolve. This solution may be stored under refrigeration, and used as long as the chromatogram obtained as directed in Assay is suitable for identifying the peaks due to the ketorolac 1-keto analog and ketorolac 1-hydroxy analog, and for the measurement of the resolu-

tion. When the procedure is run with 10 μL of this solution according to the above operating conditions, the relative retention time of ketorolac 1-hydroxy analog and ketorolac 1-keto analog to the ketorolac peak are about 0.63 and 0.89, respectively, with the resolutions of these peaks being not less than 1.5. Perform the test with 10 μL of the standard solution: the theoretical plate numbers are not less than 5500.

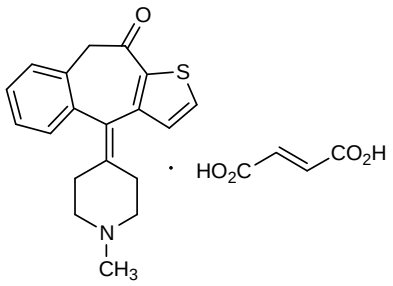
System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ketorolac is not more than 1.5 %.

Ammonium phosphate buffer—Dissolve 5.75 g of ammonium dihydrogen phosphoric acid in water to make 1000 mL, add phosphoric acid, and adjust to pH 3.0. Make adjustments, if necessary, to achieve a retention time for ketorolac of about 8 to 12 minutes.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Ketotifen Fumarate



$C_{19}H_{19}NOS \cdot C_4H_4O_4$: 425.50

(*E*)-But-2-enedioic acid; 2-(1-methylpiperidin-4-ylidene)-6-thiatricyclo[8.4.0.0^{3,7}]tetradeca-1(10),3(7),4,11,13-pentaen-8-one [*34580-14-8*]

Ketotifen Fumarate, when dried, contains not less than 99.0 % and not more than 101.0 % of ketotifen fumarate ($C_{19}H_{19}NOS \cdot C_4H_4O_4$).

Description Ketotifen Fumarate appears as white to light yellow crystalline powder.

Ketotifen Fumarate is sparingly soluble in methanol or in acetic acid (100), and slightly soluble in water, in ethanol (99.5), or in acetic anhydride.

Melting Point—About 190 °C (with decomposition).

Identification (1) Prepare the test solution with 0.03 g of Ketotifen Fumarate as directed under the Oxygen Flask Combustion Method, using 20 mL of water as the absorbing liquid: the test solution responds to the Qualitative Tests for sulfate.

(2) Determine the absorption spectra of solutions of Ketotifen Fumarate and Ketotifen Fumarate RS in methanol (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Ketotifen Fumarate and Ketotifen Fumarate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) ***Chloride***—Dissolve 0.6 g of Ketotifen Fumarate in 2.5 mL of sodium carbonate TS in a crucible, heat on a water bath to dryness, and ignite at about 500 °C. Dissolve the residue in 15 mL of water, filter if necessary, neturalize with diluted nitric acid (3 in 10), add 6 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution as directed under Chloride Limit Test. Prepare the control solution as follows: To 0.25 mL of 0.01 mol/L hydrochloric acid add 2.5 mL of sodium carbonate TS, the amount of diluted nitric acid (3 in 10) used for the neutralization of the test solution, add 6 mL of dilute nitric acid, and add water to make 50 mL (not more than 0.015 %).

(2) ***Heavy metals***—Proceed with 1.0 g of Ketitofen Fumarate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Dissolve 0.10 g of Ketitofen Fumarate in 10 mL of a mixture of methanol and ammonia TS (99 : 1), and use this solution as the test solution. To 1 mL of this solution add a mixture of methanol and ammonia TS (99 : 1) to make exactly 25 mL. To 1 mL of this solution add a mixture of methanol and ammonia TS (99 : 1) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetonitrile, water, and ammonia solution (28) (90 : 10 : 1) to a distance of about 15 cm, and air-dry the plate. Spary evenly Dragendorff's TS for spraying and hydrogen peroxide TS on the plate: the number of spots other than the principal spot obtained from the test solution is not more than 4, and the spots other than the principal spot are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

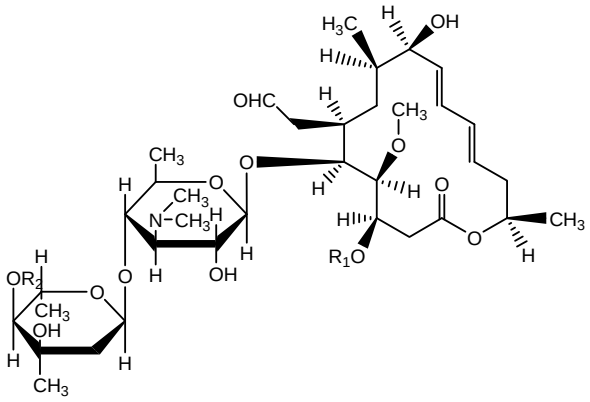
Assay Weigh accurately about 0.35 g of Ketitofen Fumarate, previously dried, dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in

Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 42.55 mg of $C_{19}H_{19}NOS \cdot C_4H_4O_4$

Containers and Storage ***Containers***—Tight containers.

Kitasamycin



Leucomycin A_1 : R_1 = H
R_2 = $COCH_2CH(CH_3)_2$
Leucomycin A_3 : R_1 = $COCH_3$
R_2 = $COCH_2CH(CH_3)_2$
Leucomycin A_4 : R_1 = $COCH_3$
R_2 = $COCH_2CH_2CH_3$
Leucomycin A_5 : R_1 = H
R_2 = $COCH_2CH_2CH_3$
Leucomycin A_6 : R_1 = $COCH_3$
R_2 = $COCH_2CH_3$
Leucomycin A_7 : R_1 = H
R_2 = $COCH_2CH_3$
Leucomycin A_8 : R_1 = $COCH_3$
R_2 = $COCH_3$
Leucomycin A_9 : R_1 = H
R_2 = $COCH_3$
Leucomycin A_{13} : R_1 = H
R_2 = $COCH_2CH_2CH_2CH_2CH_3$

(2*S*,3*S*,4*R*,6*S*)-6-{[(2*R*,3*S*,4*R*,5*R*,6*S*)-6-{[(4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-4-(acetyloxy)-10-hydroxy-5-methoxy-9,16-dimethyl-2-oxo-7-(2-oxoethyl)-1-oxacyclohexadeca-11,13-dien-6-yl]oxy}-4-(dimethylamino)-5-hydroxy-2-methyloxan-3-yl]oxy}-4-hydroxy-2,4-dimethyloxan-3-yl 3-methylbutanoate [*1392-21-8*]

Kitasamycin is a mixture of macrolide substances having antibacterial activity produced by the growth of *Streptomyces kitasatoensis*.
Kitasamycin contains not less than 1450 μg (potency) and not more than 1700 μg (potency) per mg, calculated on the anhydrous basis. The potency of Kitasamycin is expressed as the mass (potency) of kitasamycin corresponding to the amount of leucomycin A_5 ($C_{39}H_{65}NO_{14}$: 771.93), and 1 mg (potency) of kitasamycin is equivalent to 0.530 mg of leucomycin A_5 ($C_{39}H_{65}NO_{14}$).

Description Kitasamycin appears as white to pale yellowish white powder.
Kitasamycin is very soluble in acetonitrile, in methanol, or in ethanol (95), and practically insoluble in water.

Identification Determine the absorption spectrum of solutions of Kitasamycin and Leucomycin A_5 RS in methanol (1 in 40000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Water Not more than 3.0 % (0.1 g, volumetric titration, direct titration).

Content Ratio Dissolve 0.02 g of Kitasamycin in diluted acetonitrile (1 in 2) to make 20 mL, and use this solution as the test solution. Perform the test with 5 μL of the test solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amounts of leucomycin A_5, leucomycin A_4, and leucomycin A_1 by the area percentage method: 40 to 70 %, 5 to 25 %, and 3 to 12 %, respectively. The relative retention times of leucomycin A_4 and leucomycin A_1 with respect to leucomycin A_5 are 1.2 and 1.5, respectively.

Operating conditions
Detector: An ultraviolet absorption photometer (wavelength: 232 nm)
Column: A stainless steel column 4.0 mm in internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40 °C
Mobile phase: Adjust the pH of a solution of ammonium acetate (77 in 5000) to 5.5 with diluted phosphoric acid (1 in 150). To 370 mL of this solution add 580 mL of methanol and 50 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of leucomycin A_5 is about 8 minutes.
Time span of measurement: About 3 times as long as the retention time of leucomycin A_5.
System suitability
System performance: Dissolve about 20 mg of Leucomycin A_5 RS and about 20 mg of Josamycin RS in 20 mL of diluted acetonitrile (1 in 2). When the procedure is run with 5 μL of this solution under the above operating conditions, leucomycin A_5 and josamycin are eluted in this order with the resolution between these peaks being not less than 5.
System repeatability: When the test is repeated 6 times with 5 μL each of the test solution under the above operating conditions, the relative standard devia-

tion of the peak area of leucomycin A_5 is not more than 1.0 %.

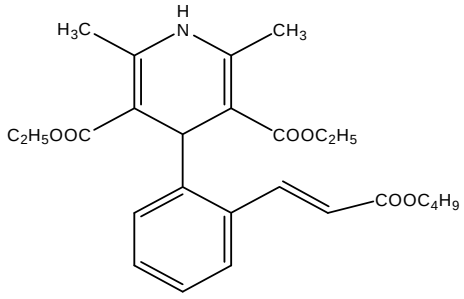
Assay ***The Cylinder-plate method*** (1) Agar media for seed and base layer- Use the culture medium in I 2 1) (1) ① under Microbial Assay for Antibiotics.

(2) Test organism- *Bacillus subtilis* ATCC 6633

(3) Weigh accurately about 30 mg (potency) of Kitasamycin, dissolve in 10 mL of methanol, and add water to make 100 mL. Pipet a suitable volume of this solution, add phosphate buffer solution (pH 8.0) so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration test solution and low concentration test solution, respectively. Separately, weigh accurately about 30 mg (potency) of Leucomycin A_5 RS, dissolve in 10 mL of methanol, add water to make 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 5 °C, and use within 3 days. Pipet a suitable volume of the standard stock solution, add phosphate buffer solution (pH 8.0) so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively. Perform the test with these solutions as directed in I 8 under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Lacidipine



$C_{26}H_{33}NO_6$: 455.54

Diethyl-2,6-dimethyl-4-[2-[(*E*)-3-[(2-methylpropan-2-yl)oxy]-3-oxoprop-1-enyl]phenyl]-1,4-dihydropyridine-3,5-dicarboxylate [*103890-78-4*]

Lacidipine contains not less than 97.5 % and not more than 102.0 % of lacidipine ($C_{26}H_{33}NO_6$), calculated on the anhydrous and solvent-free basis.

Description Lacidipine is a white to pale yellow crystalline powder.
Lacidipine is freely soluble in acetone or in dichloromethane, sparingly soluble in ethanol (99.5), and practically insoluble in water.

Melting point—About 178 °C.

Identification (1) Determine the infrared spectra of Lacidipine and Lacidipine RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) When proceed as directed in the Assay, the retention time of the principal peak in the test solution corresponds to that in the standard solution.

Purity (1) ***2-Propanol***—Weigh accurately 1.0 g of Lacidipine, add the internal standard solution to make exactly 50 mL and use this solution as the test solution. Pipet accurately 2 μL of 2-propanol, add the internal standard solution to make exactly 100 mL and use this solution as the standard solution. Perform the test with 1 μL each of the test solution and the standard solution as directed under Gas Chromatography according to the following operating conditions and calculate the content (%) of 2-propanol in the test solution (not more than 0.5 %).

Internal standard solution—Pipet accurately 2 μL of 1,4-dioxane and add dimethylacetamide to make exactly 100 mL.

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A glass column, about 0.32 mm in internal diameter and about 60 cm in length, coated inside surface with polymethylsiloxane for gas chromatography 5 μm in thickness.

Column temperature: Maintain the initial temperature of 60 °C for 1 minute, up to 110 °C at the rate of 3 °C per minute, then up to 200 °C at the rate of 50 °C per minute and hold at 200 °C for 7 minutes.

Carrier gas: Nitrogen.

Flow rate: 1.7 mL/minute.

System suitability

System performance: When the procedure is run with 1 μL of the standard solution as directed under the above operating conditions, two peaks are clearly separated with each other and the retention time for 2-propanol and 1,4-dioxane is about 6.2 minutes and about 15 minutes, respectively.

(2) ***Related substances***—Weigh accurately 10 mg of Lacidipine, add ethanol (99.5) to make exactly 10 mL, pipet 1.0 mL of this solution, add the mobile phase to make exactly 5 mL and use this solution as the test solution. Pipet 1.0 mL of the test solution, add mobile phase to make exactly 500 mL and use this solution as the standard solution. Perform the test with 20 μL of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the area of the peaks in the test solution and the standard solution the area of the peak corresponding to lacidipine related substance I obtained from the test solution is not more

than twice the area of the principal peak obtained from the standard solution (0.2 %, the relative response factor: 2), the area of any other related substance from the test solution is not more than the area of the principal peak from the standard solution (0.2 %). Calculate the content of lacidipine related substance I using the response factor and the content of any other related substance using the standard solution: total amount of related substances is not greater than 0.5 %.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with cyanosilyl silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of *n*-hexane and ethanol (99.5) (97:3). Adjust the composition so that the retention time of lacidipine is about 10 min.

Flow rate: About 2.0 mL/minute.

Water Not more than 0.2 % (0.5 g, coulometric titration).

Assay Weigh accurately 10 mg each of Lacidipine and Lacidipine RS, add ethanol (99.5) to make exactly 10 mL each, pipet 5.0 mL each, add the mobile phase to make exactly 100 mL each and use these solutions as the test solution and the standard solution, respectively. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the area of lacidipine peak in the test solution, A_T, and the standard solution, A_S.

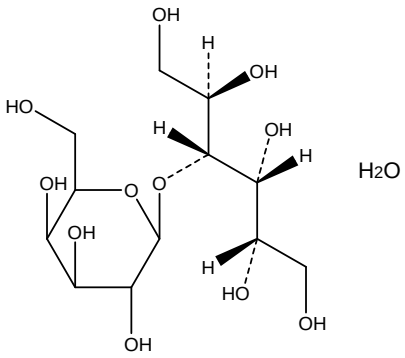
$$\text{Amount (mg) of lacidipine } (C_{26}H_{33}NO_6) = \text{Amount (mg) of Lacidipine RS} \times \frac{A_T}{A_S}$$

Operating conditions

Proceed as directed in the operating conditions of the Related Substances under the Purity.

Containers and Storage ***Containers***—Well-closed containers.

Lactitol Hydrate



$C_{12}H_{24}O_{11}\cdot H_2O$: 362.34

(2*S*,3*R*,4*R*,5*R*)-4-[(2*S*,3*R*,4*S*,5*R*,6*R*)-3,4,5-Tri-hydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-hexane-1,2,3,5,6-pentolhydrate [*81025-04-9*]

Lactitol Hydrate contains not less than 98.0 % and not more than 101.0 % of lactitol ($C_{12}H_{24}O_{11}$: 344.31), calculated on the anhydrous basis.

Description Lactitol Hydrate appears as white or pale brown crystals, is odorless and has a faint sweet taste and no aftertaste.

Identification Determine the infrared spectrum of Lactitol Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity (1) ***Heavy metals***—Proceed with 4.0 g of Lactitol Hydrate according to Method 1 and perform the test. Prepare the control solution with 2.5 mL of standard lead solution (not more than 5 ppm).

(2) ***Reducing sugars***—Dissolve about 0.5 g of Lactitol Hydrate in 2.0 mL of water and use this solution as the test solution. Separately, use 2.0 mL of 0.5 g/L glucose TS as the standard solution. Add 1 mL of alkaline copper tartrate TS to each solution at the same time, heat and allow to cool. The turbidity of the test solution is not more intense than that of the standard solution, in which a red-brown precipitate is produced (not more than 0.2 % of glucose).

(3) ***Related substances***—Weigh accurately about 1 g of Lactitol Hydrate, add water to make 100 mL and use this solution as the test solution. Separately, weigh accurately 30 mg of Lactitol RS, add water to make 100 mL and use this solution as the standard solution. Perform the test with 25 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine each peak area of each solution by the automatic integration method: the relative retention time is 0.53 for lactose, 0.58 for glucose, 0.67 for

galactose, 0.72 for lactulitol, 1.55 for galactitol and 1.68 for sorbitol, with respect to the lactitol peak from the test solution. Calculate the amount (%) of galactitol, sorbitol, lactulitol, lactose, glucose and galactose with respect to lactitol according to the following equation: the total amount of related substances is not more than 1.5 %.

$$\text{Amount (\%) of each related substance} = 100 \times \frac{C_S}{C_T} \times \frac{A_i}{A_S}$$

C: Concentration (mg/mL) of lactitol in the standard solution

C_T: Concentration (mg/mL) of Lactitol Hydrate in the test solution

A_i: Peak area of each related substance from the test solution

A_S: Peak area of lactitol from the standard solution

Operating conditions

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

Water 4.5 ~ 5.5 % (volumetric titration, direct titration).

Residue on Ignition Not more than 0.5 % (1 g).

Assay Weigh accurately about 1 g of Lactitol Hydrate, add water to make 100 mL and use this solution as the test solution. Separately, weigh accurately 0.1 g of Lactitol RS, dissolve in water to make 10 mL and use this solution as the standard solution. Perform the test with 25 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine the peak areas, A_T and A_S, of lactitol in each solution.

$$\text{Amount (mg) of lactitol (C12H24O11)} = \text{Concentration (mg/mL) of lactitol in the standard solution} \times \frac{A_T}{A_S} \times 100$$

Operating conditions

Detector: A differential refractometer

Column: A stainless steel column about 7.8 mm in internal diameter and about 30 cm in length, packed with strong acid ion-exchange resin for liquid chromatography (sulfonated styrene-divinylbenzene copolymer resin pattern, 9 μm in particle diameter).

Column temperature: A constant temperature of about 85 °C

Mobile phase: Water

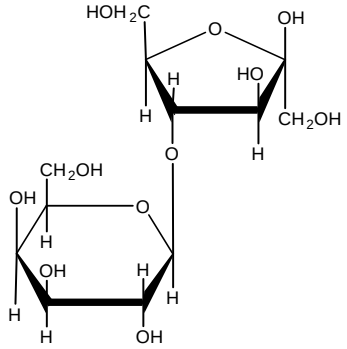
Flow rate: About 0.7 mL/minute

System suitability

System repeatability: When the test is repeated 6 times with 25 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of lactitol is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Lactulose



$C_{12}H_{22}O_{11}$: 342.30

(2*S*,3*R*,4*S*,5*R*,6*R*)-2-[(2*R*,3*S*,4*S*,5*R*)-4,5-Dihydroxy-2,5-*bis*(hydroxymethyl)oxolan-3-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol [*4618-18-2*]

Lactulose is a solution of Lactulose prepared by isomerizing lactose under the existing of alkaline and purified by ion-exchange resin.
Lactulose contains not less than 50.0 % and not more than 56.0 % of lactulose ($C_{12}H_{22}O_{11}$).

Description Lactulose is a clear, colorless of pale yellow, viscous liquid, is odorless and has a sweet taste. Lactulose is miscible with water and with formamide**.**

Identification (1) To 0.7 g of Lactulose, add 10 mL of water, 10 mL of a solution of hexaammonium heptamolybdate tetrahydrate (1 in 25) and 0.2 mL of acetic acid (100) and heat in a water-bath for 5 minutes to 10 minutes: a blue color is observed.

(2) Mix 0.3 g of Lactulose and 30 mL of water, add 16 mL of 0.5 mol/L iodine TS, then immediately add 2.5 mL of 8 mol/L sodium hydroxide TS, allow to stand for 7 minutes and add 2.5 mL of diluted sulfuric acid (3 in 20). To this solution, add a saturated solution of sodium sulfite until the solution turns pale yellow, then add 3 drops of methyl orange TS, neutralize with a solution of sodium hydroxide (4 in 25) and add water to make 100 mL. To 10 mL of this solution, add 5 mL of Fehling's TS and boil for 5 minutes: a red precipitate is produced.

Specific Gravity d_{20}^{20}: 1.320 ~ 1.360.

pH Dissolve 2.0g of Lactulose in 15 mL of water: the pH of this solution is between 3.5 and 5.5.

Purity (1) ***Heavy metals***—Proceed with 5.0 g of Lactulose according to Method 4 and perform the test. Prepare the control solution with 2.5 mL of standard lead solution (not more than 5 ppm).

(2) ***Arsenic***—Prepare the test solution with 1.0 g of Lactulose according to Method 1 and perform the test (not more than 2 ppm).

(3) ***Other saccharides***—Determine the heights of the peaks corresponding to D-galactose and lactose, respectively, on the chromatogram obtained in Assay from the test solution and the standard solution and calculate the ratios of the peak heights of D-galactose and lactose to that of the internal standard from the test solution, Q_{Ta} and Q_{Tb} and then from the standard solution, Q_{Sa} and Q_{Sb}: it contains D-galactose of not more than 11.0 % and lactose hydrate of not more than 6.0 %.

$$\text{Amount (mg) of galactose } (C_6H_{12}O_6) = \text{Amount (mg) of D-galactose} \times \frac{Q_{Ta}}{Q_{Sa}}$$

$$\text{Amount (mg) of lactose hydrate } (C_{12}H_{22}O_{11}\cdot H_2O) = \text{Amount (mg) of lactose hydrate} \times \frac{Q_{Tb}}{Q_{Sb}}$$

Loss on Drying Not more than 35.0 % (0.5 g, in vacuum, 80 °C, 5 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 1 g of Lactulose, add 10.0 mL of the internal standard solution and water to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately about 0.5 g of Lactulose RS, accurately about 80 mg of D-galactose and accurately about 40 mg of Lactose Hydrate, add 10.0 mL of the internal standard solution and water to make exactly 50 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak height of lactulose to that of the internal standard, for the test solution and the standard solution respectively.

$$\text{Amount (mg) of lactulose } (C_{12}H_{22}O_{11}) = \text{Amount (mg) of Lactulose RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of D-mannitol (1 in 20).

Operating conditions

Detector: A differential refractometer.

Column: A stainless steel column, about 8 mm in internal diameter and about 50 cm in length, packed with gel type strong acid ion-exchange resin for liquid chromatography (degree of crosslinkage: 6 %) (11 μm in particle diameter).

Column temperature: A constant temperature of about 75 °C.

Mobile phase: Water.

Flow rate: Adjust the flow rate so that the retention time of lactulose is about 18 minutes.

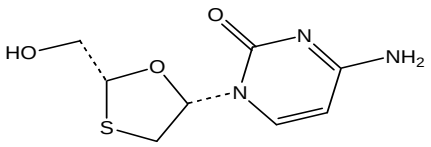
System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, lactulose and internal standard are eluted in this order with the resolution between their peaks being not less than 8.0.

System repeatability: When the test is repeated 6 times with 20μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak heights of lactulose, galactose and lactose to that of the internal standard are not more than 2.0 % respectively.

Containers and Storage ***Containers***—Tight containers.

Lamivudine



$C_8H_{11}N_3O_3S$: 229.26

4-Amino-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2-one [*134678-17-4*]

Lamivudine contains not less than 98.0 % and not more than 102.0 % of lamivudine ($C_8H_{11}N_3O_3S$), calculated on the anhydrous and solvent-free basis.

Description Lamivudine is a white solid.
Lamivudine is soluble in water.

Melting point—About 176 °C.

Identification (1) Determine the infrared spectra of Lamivudine and Lamivudine RS as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) When proceed as directed in the Lamivudine enantiomer under the Purity, the retention time of the principal peak from the test solution corresponds to that from the standard solution.

Purity (1) ***Clarity and color of solution***—Proceed with a solution of Lamivudine (1 in 20) as directed

under Infrared Spectrophotometry and measure the absorbance of this solution at 440 nm in 4 cm: not more than 0.0015.

(2) ***Heavy metals***—Transfer 1.0 g of Lamivudine to a flask, clamp the flask at an angle of about 45 °, add 8 mL of sulfuric acid and 10 mL of nitric acid and mix. Heat on low until the reaction commences, add 8 mL of sulfuric acid and 10 mL of nitric acid then raise the temperature and heat until the solution darkens. Cool, add 2 mL of nitric acid and heat again until the solution darkens. Continue heating until no further darkening occurs then heat strongly until dense white fumes are produced. Cool, add 5 mL of water, heat again until dense white fumes are produced and continue heating until the volume is reduced to 2 mL to 3 mL. Cool, add 5 mL of water and examine the color of the solution. If the color is yellow, add 1 mL of strong hydrogen peroxide, heat until dense white fumes are produced and continue heating until the volume is reduced to 2 mL to 3 mL. If the solution is still yellow in color, repeat the addition of 5 mL of water and 1 mL of strong hydrogen peroxide until the solution is colorless. Cool, dilute with water, ensuring that the total volume does not exceed 25 mL, and transfer to a 50 mL Nessler cylinder. Adjust the pH to between 3.0 and 4.0 with dilute ammonia solution then dilute with water to make 40 mL. Add 2 mL of acetate buffer solution, pH 3.5, and 1.2 mL of thioacetamide TS, mix, add water to make 50 mL and use this solution as the test solution. Separately, prepare the control solution at the same time and in the same manner as the test solution, using 2 mL of standard lead solution. Allow to stand for 2 minutes and examine the solutions against a white background: the brown color of the test solution is not more intense than that of the control solution (not more than 20 ppm).

If the result is difficult to judge, filter the solutions, slowly and at low pressure, through a membrane filter with pore size of 0.45 μm and compare the color of the filter paper.

System suitability: Prepare the blank solution in the same manner as the test solution without adding the test specimen. The control solution shows a faint brown color compared to the blank solution. Add 2 mL of standard lead solution to the test solution and use this solution as the system suitability solution. The color of the system suitability solution is of the same or greater intensity than the control solution.

(3) ***Lamivudine enantiomer***—Weigh accurately about 25 mg of Lamivudine, add water to make 100 mL and use this solution as the test solution. Perform the test with 10 μL of the test solution as directed under Liquid Chromatography according to the following operating conditions, determine the areas of the principal peaks and calculate the content of lamivudine enantiomer: not more than 0.3 %.

$$\text{Content (\%) of lamivudine enantiomer} = 100\times\frac{A_i}{A_i + A_S}$$

A_i: Peak area of lamivudine enantiomer

A_S: Peak area of lamivudine

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 270 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with beta-cyclodextrin bonded to silica gel for liquid chromatography (5-10 μm in particle diameter).

Mobile phase: A mixture of 0.1 mol/L ammonium acetate solution and methanol (95:5).

Flow rate: About 1.0 mL/minute.

System suitability

System performance: Dissolve the content of one vial of lamivudine resolution mixture I RS in 5 mL of water, wash the inner side of the vial with 2 mL each of water, combine the washings and the dissolved content, and add water to make exactly 10 mL. Perform the test with 10 μL of this solution according to the above operating conditions, the relative retention times for lamivudine peak and lamivudine enantiomer peak are about 1.0 and 1.2, respectively, with the resolution between lamivudine peak and lamivudine enantiomer peak being not less than 1.5.

0.1 mol/L Ammonium acetate solution—Dissolve about 7.7 g of ammonium acetate in water to make 1000 mL.

(4) ***Residual solvents***—Weigh accurately 5 g of Lamivudine, add 10 mL of the internal standard solution, add a mixture of water and dimethylsulfoxide (1:1) to make exactly 100 mL and use this solution as the test solution. To 10 mL of the internal standard solution, add accurately 100 μL each of ethanol (99.5), isopropyl acetate, methanol and triethylamine, add a mixture of water and dimethylsulfoxide (1:1) to make exactly 100 mL and use this solution as the standard solution. Perform the test with 0.5 μL each of the test solution and the standard solution as directed under Gas Chromatography according to the following operating conditions, determine the areas of peaks in these solutions and calculate the content of residual solvents in Lamivudine: not more than 0.2 % of ethanol, not more than 0.2 % of isopropyl acetate, not more than 0.1 % of triethylamine and not more than 0.3 % of total residual solvents.

$$\text{Content (\%) of each residual solvent} = 10\times\frac{C}{W}\times\frac{Q_i}{Q_S}$$

C: Concentration (mg/mL) of the respective analyte in the standard solution

W: Weight (g) of Lamivudine taken

Q_i: Peak area ratio of the respective analyte to the internal standard in the test solution

Q_S: Peak area ratio of the respective analyte to the

internal standard in the standard solution

Internal standard solution—Pipet 1 mL of 2-pentanone and add a mixture of dimethylsulfoxide and water (1:1) to make exactly 100 mL.

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A fused silica tube, about 0.53 mm in internal diameter and about 50 m in length, coated inside surface with dimethylpolysiloxane for gas chromatography, 5 μm in thickness.

Column temperature: Maintain the initial temperature of 70 °C for 3 minute, up to 200 °C at the rate of 30 °C per minute and hold at 200 °C for 6.5 minutes.

Carrier gas: Helium.

Injection port temperature: 150 °C.

Flow rate: 320 mL/minute.

Detector temperature: 250 °C.

(5) ***Related substances***—Weigh accurately about 25 mg of Lamivudine, dissolve in the mobile phase as directed in the Assay to make exactly 100 mL and use this solution as the test solution. Weigh accurately a portion of salicylic acid, add the mobile phase as directed in the Assay to render the concentration of 0.625 μg per mL and use this solution as the salicylic acid solution. Perform the test with 10 μL each of the test solution and the salicylic acid solution as directed under Liquid Chromatography according to the operating conditions directed in the Assay, determine the area of salicylic acid peak in these solutions and calculate the content of salicylic acid according to the equation (1): not more than 0.1 %. Calculate the content of other related substances according to the equation (2): not more than 0.3 % for the related substance having the relative retention time of about 0.4, not more than 0.2 % for the related substance having the relative retention time of about 0.9 and not more than 0.6 % for total related substances.

$$\text{Content (\%) of salicylic acid} = 10 \times \frac{C}{W} \times \frac{A_T}{A_S} \quad (1)$$

C: Concentration (μg/mL) of salicylic acid in the salicylic acid solution

W: Weight (mg) of Lamivudine taken

A_T: Peak area of salicylic acid obtained from the test solution

A_T: Peak area of salicylic acid obtained from the salicylic acid solution

$$\text{Content (\%) of other related substance} = 100 \times \frac{A_i}{A_S} \quad (2)$$

A_i: Peak area of related substance other than salicylic acid obtained from the test solution

A_S: Total area of peaks obtained from the test solution

Water Not more than 0.2 % (1 g, coulometric titration).

Assay Weigh accurately about 25 mg each of Lamivudine and Lamivudine RS, add a mixture of 0.025 mol/L ammonium acetate buffer and methanol (95:5) to make exactly 100 mL each and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the area of the principal peak in the test solution, A_T, and in the standard solution, A_S.

$$\text{Amount (mg) of lamivudine } (C_8H_{11}N_3O_3S) = \text{Amount (mg) of Lamivudine RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 277 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3-10 μm in particle diameter).

Mobile phase: A mixture of 0.025 mol/L ammonium acetate buffer and methanol (95:5).

Column temperature: A constant temperature of about 35 °C.

Flow rate: About 1.0 mL/minute.

System suitability

System performance: Dissolve the content of one vial of lamivudine resolution mixture II RS in 5 mL of the mobile phase. When the procedure is run with 10 μL of this solution as directed under the above operating conditions, the relative retention times for lamivudine peak and lamivudine enantiomer peak are about 1.0 and 0.9, respectively, with the resolution between lamivudine peak and lamivudine enantiomer peak being not less than 1.5.

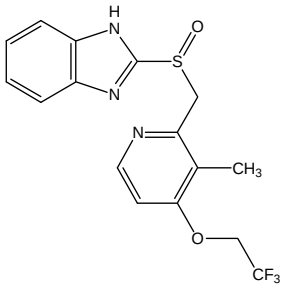
System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the areas of lamivudine peak is not more than 2.0 %.

0.025 mol/L Ammonium acetate buffer—Weigh about 1.9 g of ammonium acetate, dissolve in 900 mL of water and adjust the pH to 3.8 ± 0.2 with acetic acid and add water to make 1000 mL.

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Lansoprazole



$C_{16}H_{14}F_3N_3O_2S$: 369.36

2-[[3-Methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]methylsulfinyl]-1*H*-benzimidazole [*103577-45-3*]

Lansoprazole contains not less than 99.0 % and not more than 101.0 % of lansoprazole ($C_{16}H_{14}F_3N_3O_2S$), calculated on the anhydrous basis.

Description Lansoprazole is a white or brown crystalline powder.

Lansoprazole is freely soluble in *N,N*-dimethylformamide, soluble in methanol, sparingly soluble in ethanol (99.5), very slightly soluble in ether and practically insoluble in water.

Melting point—About 166 °C (with decomposition)

Identification (1) Determine the absorption spectra of solutions of Lansoprazole and Lansoprazole RS in methanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Lansoprazole and Lansoprazole RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity ***Related substances***—Weigh accurately about 0.125 g of Lansoprazole, dissolve in methanol to make exactly 50 mL, pipet 1.0 mL of this solution, add a mixture of the mobile phase A and the mobile phase B (9:1) to make exactly 10 mL and use this solution as the test solution. Prepare the test solution immediately before use. Separately, weigh accurately about 25 mg of Lansoprazole RS, dissolve in methanol to make exactly 100 mL, pipet 5.0 mL of this solution, add methanol to make exactly 50 mL. To 1.0 mL of this solution, add a mixture of the mobile phase A and the mobile phase B (9:1) to make exactly 10 mL and use this solution as the standard solution. To 9 mL of a mixture of the mobile phase A and the mobile phase B (9:1), add 1 mL of methanol and use this solution as the blank solution. Perform the test with 40 μL each of the test solution, the standard solution and the blank solution as directed in the peak area percentage method under Liquid Chromatography. Disregard the peaks obtained from the test solution corresponding to the peaks obtained from the blank solution and determined the peak area of each related substance other than the principal peak from the test solution, A_i, and the peak area of the principal peak from the standard solution, A_S: total content of related substances is not more than 0.1 %.

$$\text{Content (\%) of each related substance} = 50 \times \frac{C}{W} \times \frac{A_i}{A_S}$$

C: Concentration (μg/mL) of lansoprazole in the standard solution

W: Weight (mg) of Lansoprazole taken

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: Water

Mobile phase B: A mixture of acetonitrile, water and triethylamine (160:40:1). Adjust the pH of the solution to 7.0 by the addition of phosphoric acid.

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-40	90→20	10→80
40-50	20	80
50-51	20→90	80→10
51-60	90	10

Flow rate: 0.8 mL/min.

System suitability

System performance: Weigh 5 mg each of Lansoprazole RS and lansoprazole related substance I {2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl] sulfonyl]benzimidazole} RS, dissolve in methanol to make 200 mL, pipet 1.0 mL of this solution and add a mixture of the mobile phase A and the mobile phase B (9:1) to make exactly 10 mL. When the procedure is run with 40 μL of this solution under the above operating conditions, the resolution between lansoprazole peak and the related substance I peak is not less than 6.

System repeatability: Weigh 2.5 mg of lansoprazole related substance I RS, dissolve in methanol to make 100 mL, pipet 1.0 mL of this solution and add a mixture of the mobile phase A and the mobile phase B (9:1) to make exactly 10 mL. When the test is repeated 6 times with 40 μL each of this solution under the above operating conditions, the relative standard deviation of the areas of lansoprazole related substance I peak is not more than 3 %.

Water Not more than 0.1 % (1.0 g, volumetric titration, direct titration). Use 50 mL of a mixture of pyridine and ethylene glycol (9:1 or 8:2) as the solvent in this test.

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 50 mg each of Lansoprazol and Lansoprazole RS, dissolve in the internal standard solution to make 10 mL each, pipet 1 mL each of these solution, add a mixture, prepared by mixing water, acetonitrile and triethylamine (60:40:1) and by adjusting the pH of the solution to 10 with phosphoric acid, to make exactly 50 mL each and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determined the ratios of the peak area of lansoprazole to that of the internal standard from the test solution, Q_T, and from the standard solution, Q_S.

$$\text{Amount (mg) of lansoprazole } (C_{16}H_{14}F_3N_3O_2S) = \text{Amount (mg) of Lansoprazole RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—Weigh about 25 mg of 4'-ethoxyacetophenone and dissolve in a mixture, prepared by mixing water, acetonitrile and triethylamine (60:40:1) and by adjusting the pH of the solution to 10 with phosphoric acid, to make 10 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of water, acetonitrile and triethylamine (60:40:1). Adjust the pH of the solution to 7.0 by the addition of phosphoric acid.

Flow rate: 1 mL/min.

System suitability

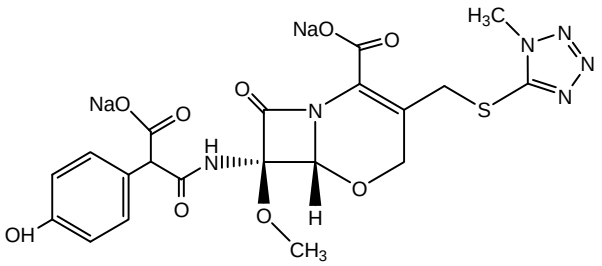
System performance: Weigh 5 mg each of Lansoprazole RS and lansoprazole related substance I RS, and dissolve in a mixture, prepared by mixing water, acetonitrile and triethylamine (60:40:1) and by adjusting the pH of the solution to 10 with phosphoric acid, to make exactly 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between lansoprazole peak and the related substance I peak is not less than 5.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the areas of lansoprazole peak is not more than 0.5 %.

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Latamoxef Sodium



$C_{20}H_{18}N_6Na_2O_9S$: 564.44

Disodium (6*R*,7*R*)-7-{[carboxy(4-hydroxyphenyl)acetyl]amino}-7-methoxy-3-{[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl}-8-oxo-5-oxa-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [*64953-12-4*]

Latamoxef Sodium contains not less than 830 μg (potency) and not more than 940 μg (potency) per mg of latamoxef ($C_{20}H_{18}N_6O_9S$: 520.47), calculated on the anhydrous basis.

Description Latamoxef Sodium appears as white to pale yellow powder or masses.

Latamoxef Sodium is very soluble in water, freely soluble in methanol, and slightly soluble in ethanol (95).

Identification (1) Determine the absorption spectra of the solutions of Latamoxef Sodium and Latamoxef Sodium RS (3 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Latamoxef Sodium and Latamoxef Sodium RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Determine the ^1H, the spectrum of a solution of Latamoxef Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10), as directed under Nuclear Magnetic Resonance Spectroscopy, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A and B, at around δ 3.5 ppm and at around δ 4.0 ppm. The ratio of the integrated intensity of these signals, A:B is about 1:1.

(4) Latamoxef Sodium responds to the Qualitative Tests (1) for sodium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: -32 ~ -40° (0.5 g calculated on the anhydrous basis, phosphate buffer solution , pH 7.0, 50 mL, 100 mm).

Absorbance $E_{1\,cm}^{1\%}$ (270 nm): 200 ~ 230 (30 mg calculated on the anhydrous basis, water, 1000 mL).

pH The pH of a solution obtained by dissolving 1.0 g (potency) of Latamoxef Sodium in 10 mL of water is between 5.0 and 7.0.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Latamoxef Sodium in 10 mL of water: the solution is clear and has no more color than the following control solution.

Control solution—To a mixture of 3 mL of cobalt (II) chloride hexahydrate stock CS and 36 mL of iron (III) chloride hexahydrate stock CS, add 11 mL of diluted hydrochloric acid (1 in 10). To 2.5 mL of this solution, add 7.5 mL of diluted hydrochloric acid (1 in 10).

(2) ***Heavy metals***—Carbonize 1.0 g of Latamoxef Sodium by heating gently, previously powdered if it is in masses. After cooling, add 10 mL of a solution of magnesium nitrate in ethanol (95) (1 in 10) and ignite the ethanol to burn. Cool, add 1 mL of sulfuric acid and proceed according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Prepare the test solution by dissolving 1.0 g of Latamoxef Sodium in 20 mL of water and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve an amount of Latamoxef Sodium, equivalent to about 25 mg (potency), in water to make exactly 50 mL, and use this solution as the test solution. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the peak area of 1-methyl-1*H*-tetrazole-5-thiol, having a relative retention time of about 0.5 with respect to the first eluted peak of the two peaks of latamoxef obtained from the test solution, is not greater than the peak area of latamoxef from the standard solution, and the peak area of decarboxylatamoxef having the relative retention time of about 1.7 with respect to the first peak of the two peaks of Latamoxef, is not greater than 2 times that of latamoxef from the standard solution. For this ccalculation, use the peak area for 1-methyl-1*H*-tetrazole-5-thiol after multiplying by its response factor, 0.52.

Operating conditions

Proceed as directed in the operating conditions in the Assay.

System suitability

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of latamoxef is not more than 2.0 %.

Water Not more than 5.0 % (0.5 g, volumetric titration, back titration).

Sterility Test It meets the requirement, when Latamoxef Sodium is used in a sterile preparation.

Bacterial Endotoxins Less than 0.0125 EU/mg (potency) of latamoxef, when Latamoxef Sodium is used in a sterile preparation.

Isomer Ratio Dissolve 25 mg of Latamoxef Sodium in water to make 50 mL, and use this solution as the test solution. Perform the test with 5 μL of the test solution as directed under Liquid Chromatography according to the following operating conditions, and determine the areas, A_a and A_b, of the two peaks in order of elution, which appear close to each other at the retention time of about 10 minutes: A_a/A_b is between 0.8 and 1.4.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column 4 mm in internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 7.7 g of ammonium acetate in water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of the first eluted peak of the two peaks of latamoxef is about 8 minutes.

System suitability

System performance: When the procedure is run with 5 μL of the test solution under the above operating conditions, the resolution between the two peaks of latamoxef is not less than 3.

System repeatability: When the test is repeated 3 times with 5 μL each of the test solution under the above operating conditions, the relative standard deviation of the area of the first eluted peak of the two peaks of latamoxef is not more than 2.0 %.

Assay Weigh accurately an amount of Latamoxef Sodium and Latamoxef Sodium RS, equivalent to about 25 mg (potency) each, dissolve in exactly 5 mL

of the internal standard solution, add water to make exactly 50 mL, and use these solutions as the test solution and the standard solution. Perform the test with 5 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of latamoxef to that of the internal standard.

$$\text{Amount [μg (potency)] of latamoxef } (C_{20}H18N_6O_9S) = \text{Amount [μg (potency)] of Latamoxef Sodium RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of *m*-cresol (3 in 200)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength 254 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter)

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 6.94 g of potassium dihydrogen phosphate, 3.22 g of disodium hydrogen phosphate dodecahydrate and 1.60 g of tetra *n*-butylammonium bromide in water to make exactly 1000 mL. To 750 mL of this solution add 250 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of latamoxef is about 7 minutes.

System suitability

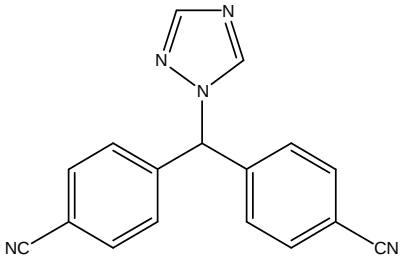
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, latamoxef and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of latamoxef to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Not exceeding 5 °C.

Letrozole



$C_{17}H_{11}N_5$: 285.30

4-[(4-Cyanophenyl)-(1,2,4-triazol-1-yl)methyl]benzonitrile [*112809-51-5*]

Letrozole contains not less than 98.0 % and not more than 102.0 % of letrozole ($C_{17}H_{11}N_5$), calculated on the anhydrous basis.

Description Letrozole appears as white to pale yellow crystals or crystalline powder.

Letrozole is freely soluble in dichloromethane, slightly soluble in ethanol (95) and practically insoluble in water.

Identification (1) Determine the infrared spectra of Letrozole and Letrozole RS as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) When proceed as directed in the Assay, the retention time of the principal peak from the test solution corresponds to that from the standard solution.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Letrozole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Weigh accurately about 25 mg of Letrozole, dissolve in 75 mL of acetonitrile, add water to make exactly 250 mL and use this solution as the test solution. Separately, weigh accurately 10 mg of Letrozole RS, dissolve in 30 mL of acetonitrile and add water to make exactly 100 mL. To 5.0 mL of this solution, add a mixture of water and acetonitrile (7:3) to make exactly 50 mL, pipet 5.0 mL of this solution, add a mixture of water and acetonitrile (7:3) to make exactly 50 mL and use this solution as the standard solution. Perform the test with 15 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the areas of peaks from these solutions: not more than 0.3 % for letrozole related substance I [4,4'-(1*H*-1,3,4-triazol-1-ylmethylene) dibenzonitrile], not more than 0.2 % for 4,4',4''-methylide netrisbenzonitrile, not more than 0.1 % for any other related substance and not more than 0.3 % for total related substances (the relative

retention time for 4,4',4''-methylidenetrisbenzonitrile is about 2.4).

$$\text{Content (\%) of each related substance} = \frac{A_i}{A_S} \times \frac{C_S}{C_T}$$

A_i: Area of each related substance peak obtained from the test solution

A_S: Area of the principal peak obtained from the standard solution

C_S: Concentration (mg/L) of letrozole in the standard solution

C_T: Concentration (mg/L) of letrozole in the test solution

Operating conditions

Detector, column, mobile phase and flow rate: proceed as directed in the operating conditions under Assay

System suitability

System performance: Weigh 2 mg of letrozole related substance I RS and 10 mg of Letrozole RS, dissolve in 30 mL of acetonitrile, add water to make 100 mL, pipet 5.0 mL of this solution and add a mixture of water and acetonitrile (7:3) to make 50 mL. When the procedure is run with 15 μL of this solution under the above operating conditions, the relative retention times are about 0.67 and 1.0 for letrozole related substance I and letrozole, respectively, with the resolution between the two peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 15 μL each of the standard solution under the above operating conditions, the relative standard deviation of the areas of letrozole peak is not more than 10.0 %.

Water Not more than 0.3 % (1 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 20 mg each of Letrozole and Letrozole RS, dissolve 30 mL of acetonitrile, add water to make exactly 100 mL each, pipet 5.0 mL each of these solutions, add a mixture of water and acetonitrile (7:3) to make exactly 100 mL each and use these solution as the test solution and the standard solutions, respectively. Perform the test with 20 μL each with the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the area of letrozole peak in the test solution, A_T, and in the standard solution, A_S.

$$\text{Amount (mg) of letrozole } (C_{17}H_{11}N_5) = \text{Amount (mg) of Letrozole RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 12.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: Water

Mobile phase B: Acetonitrile

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0	70	30
0-25	70→30	30→70

Flow rate: 1.0 mL/min.

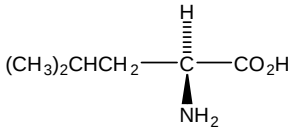
System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the symmetry factor for letrozole peak is between 0.8 and 1.5.

System repeatability: When the test is repeated 5 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the areas of letrozole peak is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

L-Leucine



$C_6H_{13}NO_2$: 131.17

(2*S*)-2-Amino-4-methylpentanoic acid [*61-90-5*]

L-Leucine, when dried, contains not less than 98.5 % and not more than 101.0 % of L-leucine ($C_6H_{13}NO_2$).

Description L-Leucine appears as white crystals or crystalline powder, is odorless or has a faint characteristic odor and has a slightly bitter taste.

L-Leucine is freely soluble in formic acid, sparingly soluble in water and practically insoluble in ethanol (95).

L-Leucine dissolves in dilute hydrochloric acid.

Identification Determine the infrared spectra of L-Leucine and L-Leucine RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit simi-

lar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +14.5 ~ +16.0° (after drying, 1 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH Dissolve 1.0 g of L-Leucine in 100 mL of water: the pH of this solution is between 5.5 and 6.5.

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of L-Leucine in 10 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) ***Chloride***—Dissolve 0.5 g of L-Leucine in 40 mL of water and 6 mL of dilute nitric acid, add water to make 50 mL, and perform the test. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021 %).

(3) ***Sulfate***—Dissolve 0.6 g of L-Leucine in 40 mL of water and 1 mL of dilute hydrochloric acid, add water to make 50 mL, and perform the test. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028 %).

(4) ***Ammonium***—Perform the test with 0.25 g of L-Leucine. Prepare the control solution with 5.0 mL of standard ammonium solution (not more than 0.02 %).

(5) ***Heavy metals***—Proceed with 1.0 g of L-Leucine according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(6) ***Iron***—Dissolve 0.333 g of L-Leucine in water to make 45 mL, add 2 mL of hydrochloric acid and use this solution as the test solution. To 1.0 mL of iron standard solution, add water to make 45 mL, add 2 mL of hydrochloric acid and use this solution as the standard solution. To each of the test solution and the standard solution, add 50 mg of ammonium peroxydisulfate and 3 mL of 30 % ammonium thiocyanate solution: the color obtained from the test solution is not more intense than that from the standard solution (not more than 30 ppm).

(7) ***Arsenic***—Prepare the test solution with 1.0 g of L-Leucine according to Method 2 and perform the test (not more than 2 ppm).

(8) ***Related substances***—Dissolve 0.1 g of L-Leucine by warming, after cooling, add water to make 25 mL and use this solution as the test solution. Proceed as directed in the Purity (7) under L-Isoleucine.

Loss on Drying Not more than 0.3 % (1 g, 105 °C, 3 hours).

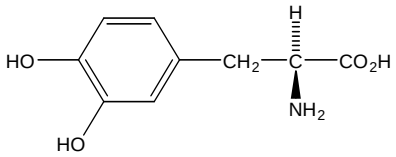
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.13 g L-Leucine, previously dried and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 13.117 mg of $C_6H_{13}NO_2$

Containers and Storage ***Containers***—Well-closed containers.

Levodopa



$C_9H_{11}NO_4$: 197.19

(2*S*)-2-Amino-3-(3,4-dihydroxyphenyl)propanoic acid [*59-92-7*]

Levodopa, when dried, contains not less than 98.5 % and not more than 101.0 % of levodopa ($C_9H_{11}NO_4$).

Description Levodopa appears as white or pale grayish white crystals or crystalline powder and is odorless.
Levodopa is freely soluble in formic acid, slightly soluble in water and practically insoluble in ethanol (95) or in ether.
Levodopa dissolves in dilute hydrochloric acid.

pH—The pH of a saturated solution of Levodopa is between 5.0 and 6.5.

Melting point—About 275 °C (with decomposition).

Identification (1) Take 5 mL of a solution of Levodopa (1 in 1000), add 1 mL of ninhydrin TS and heat for 3 minutes in a water-bath: a purple color is observed.

(2) Take 2 mL of a solution of Levodopa (1 in 5000), add 10 mL of 4-aminoantipyrine TS and shake: a red color is observed.

(3) Dissolve separately 3 mg each of Levodopa and Levodopa RS in 0.001 mol/L hydrochloric acid TS to make 100 mL each and determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Specific Optical Rotation $[\alpha]_D^{20}$: -11.5 ~ -13.0° (after drying, 2.5 g, 1 mol/L hydrochloric acid TS, 50 mL, 100 mm).

Absorbance $E_{1\,cm}^{1\%}$ (280 nm): 136 ~ 146 (after drying, 30 mg, 0.001 mol/L hydrochloric acid TS, 1000 mL).

Purity (1) ***Clarity and color of solution***—Dissolve

1.0 g of Levodopa in 20 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) ***Chloride***—Dissolve 0.5 g of Levodopa in 6 mL of dilute nitric acid and add water to make 50 mL. Perform the test. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021 %).

(3) ***Sulfate***—Dissolve 0.40 g of Levodopa in 1 mL of dilute hydrochloric acid and add water to make 50 mL. Perform the test. Prepare the control solution with 0.25 mL of 0.005 mol/L sulfuric acid VS (not more than 0.030 %).

(4) ***Heavy metals***—Proceed with 1.0 g of Levodopa according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(5) ***Arsenic***—Prepare the test solution with 1.0 g of Levodopa in 5 mL of dilute hydrochloric acid and perform the test (not more than 2 ppm).

(6) ***Related substances***—Dissolve 0.10 g of Levodopa in 10 mL of sodium metabisulfite TS and use this solution as the test solution. Pipet 1.0 mL of the test solution, add sodium metabisulfite TS to make exactly 25 mL. Pipet 1.0 mL of this solution, add sodium metabisulfite TS to make exactly 20 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, acetic acid (100) and methanol (10 : 5 : 5 : 1) to a distance of about 10 cm and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate and heat at 90 °C for 10 minutes: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.3 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

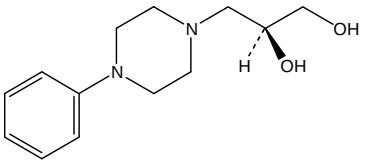
Assay Weigh accurately about 0.3 g Levodopa, previously dried and dissolve in 3 mL of formic acid, add 80 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue-green to green (indicator: 3 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 19.719 mg of $C_9H_{11}NO_4$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Levodropropizine



$C_{13}H_{20}N_2O_2$: 236.31

(2*S*)-3-(4-Phenylpiperazin-1-yl)propane-1,2-diol
[*99291-25-5*]

Levodropropizine, when dried, contains not less than 98.5 % and not more than 101.0 % of levodropropizine ($C_{13}H_{20}N_2O_2$).

Description Levodropropizine appears as white powder.
Levodropropizine is freely soluble in dilute acetic acid or in methanol, and slightly soluble in water or in ethanol (95).

Identification Determine the infrared spectra of Levodropropizine and Levodropropizine RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

pH To 2.5 g of Levodropropizine, add 100 mL of water, heat to dissolve and cool: the pH of this solution is between 9.2 and 10.2.

Specific Optical Rotation $[\alpha]_D^{20}$: -30.0 ~ –33.5° (1.5 g, after drying, 21 mg/mL, hydrochloric acid 50 mL, 100 mm).

Purity (1) ***Related substance I and related substances***—Dissolve 24.0 mg of Levodropropizine in the mobile phase to make exactly 100 mL and use this solution as the test solution. Separately, dissolve 12.0 mg of levodropropizine related substance I (1-phenylpiperazine) in methanol to make exactly 100 mL, pipet 1.0 mL of this solution, add the mobile phase to make exactly 100 mL and use this solution as the standard solution (1). Pipet 0.5 mL of the test solution and 1.0 mL of the standard solution (1), mix, add the mobile phase to make exactly 100 mL and use this solution as the standard solution (2). Perform the test with 20 μL each of the test solution, the standard solutions (1) and (2) as directed under Liquid Chromatography according to the following operating condition, and determine the area of the peaks in the test solution and the standard solutions: the area of the peak corresponding to the related substance I obtained from the test solution is not more than the area of the corresponding peak obtained from the standard solution (1) (0.5 %) and the individual area of any other peak from the test solution is not more than 0.2 times the area of

the related substance I peak from the standard solution (1) (0.1 %). Disregard any peaks having not more than 0.02 times the area of the related substance I peak from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 10 cm in length, packed with base-deactivated octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of phosphate buffer and methanol (88:12).

Flow rate: 1.5 mL/minute.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution (2) under the above operating conditions, the resolution between levodropropizine peak and the related substance I peak is not less than 2.0.

Phosphate buffer—Dissolve 6.81 g of potassium dihydrogen phosphate in water to make 1000 mL and adjust the pH to 3.0 by the addition of phosphoric acid.

(2) ***Related substance II***—Prepare immediately before use. Dissolve 0.50 g of Levodropropizine in dichloromethane to make exactly 2.5 mL and use this solution as the test solution. Separately, dissolve 0.20 g of levodropropizine related substance II {[2*RS*)-oxiran-2-yl]methanol (glycidol)} in dichloromethane to make exactly 100 mL, pipet 0.5 mL of this solution, add dichloromethane to make exactly 100 mL and use this solution as the standard solution (1). Dissolve 0.50 g of Levodropropizine in a volume of dichloromethane, add 0.5 mL of the standard solution (1), add dichloromethane to make exactly 2.5 mL and use this solution as the standard solution (2). Perform the test with 1 μL of the test solution and the standard solution (2) as directed under Gas Chromatography according to the following operating conditions, and determine the area of the peaks from the test solution and the standard solution (2): the area of the peak corresponding to the related substance II obtained from the test solution is not more than 0.5 times the area of the peak corresponding to the related substance II obtained from the standard solution (2) (0.001 %).

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A glass column, about 0.53 mm in internal diameter and about 30 m in length, coated inside surface with poly[(cyanopropyl) (phenyl)] [dimethyl] siloxane for gas chromatography 3 μm in thickness.

Injection port temperature: 170 °C.

Detector temperature: 250 °C.

Carrier gas: Helium.

Flow rate: 2.5 mL/minute.

Split ratio: About 1 : 8

(3) ***Enantiomer***—Dissolve 10.0 mg of Levodropropizine in 10.0 mL of a mixture of hexane and ethanol (6:4), pipet 1.0 mL of this solution, add a mixture of hexane and ethanol (6:4) to make exactly 50 mL, and use this solution as the test solution. Separately, dissolve 10.0 mg of Levodropropizine RS in 10.0 mL of a mixture of hexane and ethanol (6:4), pipet 1.0 mL of this solution, add a mixture of hexane and ethanol (6:4) to make exactly 50 mL, and use this solution as the standard solution (1). Dissolve 10.0 mg of levodropropizine related substance III [(2*R*)-3-(4-phenyl piperazin-1-yl)propane-1,2-diol (dextrodropropizine)] RS in 10.0 mL of hexane and ethanol (6:4), pipet 1 mL of this solution, add a mixture of hexane and ethanol (6:4) to make exactly 50 mL and use this solution as the standard solution (2). Pipet 1.0 mL of the standard solution (2), add a mixture of hexane and ethanol (6:4) to make exactly 50 mL and use this solution as the standard solution (3). Mix 1 mL of the standard solution (1) and 1 mL of the standard solution (2), and use this mixture as the standard solution (4). Perform the test with 20 μL each of the test solution, the standard solution (1), (3) and (4) as directed under Liquid Chromatography according to the following operating conditions and determine the area of the peaks in the solutions: the area of the peak corresponding to the related substance III obtained from the test solution is not more than the area of the corresponding peak obtained from the standard solution (3) (not more than 2 %).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with silica gel OD for chiral separations for liquid chromatography.

Mobile phase: A mixture of hexane, ethanol and diethylamine (95:5:0.2).

Flow rate: 0.8 mL/minute.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution (4) under the above operating conditions, the related substance III and levodropropizine are eluted in this order with the resolution between these two peaks being not less than 2.0.

Loss on Drying Not more than 0.5 % (0.5 g, 0.15-0.25 kPa, 60 °C, P_2O_5, 4 hours).

Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 0.2 g of Levodropropizine, add 50 mL of acetic acid (100) to dissolve and titrate with 0.1 mol/L of perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Read the volume added at the second point

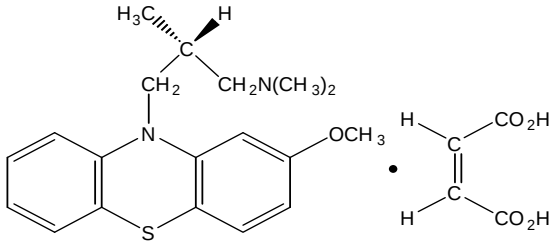
of inflexion. Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 11.82 mg of $C_{13}H_{20}N_2O_2$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Levomepromazine Maleate



$C_{19}H_{24}N_2OS \cdot C_4H_4O_4$: 444.54

(*Z*)-But-2-enedioic acid;(2*R*)-3-(2-methoxy phenothiazin-10-yl)-*N*,*N*,2-trimethylpropan-1-amine [*7104-38-3*]

Levomepromazine Maleate, when dried, contains not less than 98.0 % and not more than 101.0 % of levomepromazine maleate ($C_{19}H_{24}N_2OS \cdot C_4H_4O_4$).

Description Levopromazine Maleate appears as white crystals or crystalline powder, is odorless and has a slightly bitter taste.

Levopromazine Maleate is freely soluble in acetic acid (100), soluble in chloroform, sparingly soluble in methanol, slightly soluble in ethanol (95) or in acetone, very slightly soluble in water and practically insoluble in ether.

Melting point—184 ~ 190 °C (with decomposition).

Identification (1) Dissolve 5 mg of Levopromazine Maleate in 5 mL of sulfuric acid: a red-purple color is observed, which slowly becomes deep red-purple. To this solution, add 1 drop of potassium dichromate TS: brownish orange color is observed.

(2) Take 0.2 g of Levopromazine Maleate, add 5 mL of sodium hydroxide TS and 20 mL of ether and shake well. Separate the ether layer, wash twice with 10 mL volumes of water, add 0.5 g of anhydrous sodium sulfate, filter, evaporate the ether solution on a water-bath and dry the residue at 105 °C for 2 hours: the residue melts between 124 °C and 128 °C.

(3) Take 0.5 g of Levopromazine Maleate, add 5 mL of water and 2 mL of ammonia solution (28), extract with three 5 mL volumes of chloroform, separate and evaporate the water layer to dryness. To the residue, add 2 to 3 drops of dilute sulfuric acid and 5 mL of water and extract with four 25 mL volumes of ether. Combine all the ether extracts, evaporate the ether on a water-bath at a temperature of about 35 °C with the aid of a current of air: the residue melts between 128 °C and 136 °C.

Specific Optical Rotation $[\alpha]_D^{20}$: -13.5 ~ -16.5° (after drying, 0.5 g, chloroform, 20 mL, 200 mm).

Purity (1) ***Clarity and color of solution***—Take 0.5 g of Levopromazine Maleate, add 10 mL of methanol and dissolve by warming: the solution is clear and colorless to pale yellow.

(2) ***Chloride***—Dissolve 0.5 g of Levopromazine Maleate in 40 mL of methanol and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS, 40 mL of methanol, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.028 %).

(3) ***Heavy metals***—Proceed with 2.0 g of Levopromazine Maleate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

Loss on Drying Not more than 0.5 % (2 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

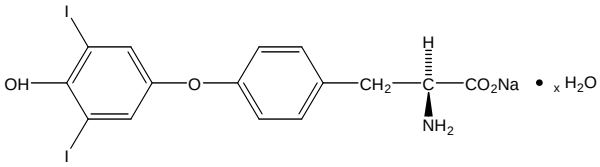
Assay Weigh accurately about 1.0 g of Levopromazine Maleate, previously dried and dissolve in a mixture of 40 mL of acetic acid (100) and 20 mL of acetone for nonaqueous titration. Titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from red-purple through blue-purple to blue (indicator: 5 drops of bromocresol green-methyl rosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 44.45 mg of $C_{19}H_{24}N_2OS \cdot C_4H_4O_4$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Levothyroxine Sodium Hydrate



$C_{15}H_{10}I_4NNaO_4 \cdot xH_2O$

Sodium(2*S*)-2-amino-3-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]propanoate hydrate [*25416-65-3*]

Levothyroxine Sodium Hydrate contains not less than 97.0 % and not more than 101.0 % of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$: 798.85), calculated on the dried basis.

Description Levothyroxine Sodium Hydrate is a pale yellow to pale yellow-brown powder and is odorless.
Levothyroxine Sodium Hydrate is slightly soluble in ethanol (95) and practically insoluble in water or in ether.
Levothyroxine Sodium dissolves Hydrate in sodium hydroxide TS.
Levothyroxine Sodium Hydrate is gradually affected by light.

Identification (1) Heat 0.1 g of Levothyroxine Sodium Hydrate over a flame: a purple gas evolves.

(2) Take 0.5 mg of Levothyroxine Sodium Hydrate, add 8 mL of a mixture of water, ethanol, hydrochloric acid and sodium hydroxide TS (6 : 5 : 2 : 2), warm in a water-bath for 2 minutes, cool and add 0.1 mL of sodium nitrite TS. Allow to stand in a dark place for 20 minutes and add 1.5 mL of ammonia solution (28): a yellowish red color is observed.

(3) Determine the absorption spectra of solutions of Levothyroxine Sodium Hydrate and Levothyroxine Sodium Hydrate RS in dilute sodium hydroxide TS (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Moisten Levothyroxine Sodium Hydrate with sulfuric acid and ignite: the residue responds to the Qualitative Tests (1) and (2) for sodium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: -5 ~ -6° (0.3 g, calculated on the dried basis, a mixture of ethanol (95) and sodium hydroxide TS (2 : 1), 10 mL, 100 mm).

Purity (1) ***Clarity and color of solution***—Dissolve 0.3 g of Levothyroxine Sodium Hydrate in 10 mL of a mixture of ethanol (95) and sodium hydroxide TS (2 : 1) by warming: the solution is clear and pale yellow to pale yellow-brown in color.

(2) ***Soluble halides***—Dissolve 10 mg of Levothyroxine Sodium Hydrate in 10 mL of water and 1 drop of dilute nitric acid, shake for 5 minutes and filter. To the filtrate, add water to make 10 mL, then add 3 drops of silver nitrate TS and mix: the solution has no more opalescence than the following control solution.

Control solution—To 0.20 mL of 0.01 mol/L hydrochloride VS, add 10 mL of water and 1 drop of dilute nitric acid and proceed as directed above.

(3) ***Related substances***—Dissolve 20 mg of Levothyroxine Sodium Hydrate in 2 mL of a mixture of ethanol (95) and ammonia solution (28) (14 : 1) and use this solution as the test solution. Pipet 1.0 mL of this solution, add a mixture of ethanol (95) and ammonia solution (28) (14 : 1) to make exactly 50 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of *t*-butyl alcohol, *t*-amyl alcohol, water, ammonia solution (28) and 2-butanone (59 : 32 : 17 : 15 : 7) to a distance of about 12 cm and air-dry the plate. Spray evenly a solution of 0.3 g of ninhydrin in 100 mL of a mixture of 1-butanol and acetic acid (100) (97 : 3) on the plate and heat at 100 °C for 3 minutes: the red-purple spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying 7.0 ~ 11.0 % (0.5 g, in vacuum, P_2O_5, 60 °C, 4 hours).

Assay Weigh accurately about 25 mg of Levothyroxine Sodium Hydrate and proceed as directed in the Assay under Liothyronine Sodium.

Each mL of 0.02 mol/L sodium thiosulfate VS
= 0.6657 mg of $C_{15}H_{10}I_4NNaO_4$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Levothyroxine Sodium Tablets

Levothyroxine Sodium Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$: 798.85).

Method of Preparation Prepare as directed under Tablets, with Levothyroxine Sodium Hydrate.

Identification (1) Weigh a portion of powdered Levothyroxine Sodium Tablets, equivalent to 0.5 mg of Levothyroxine Sodium according to the labeled amount, add 8 mL of a mixture of water, ethanol, hydrochloric acid and sodium hydroxide TS (6 : 5 : 2 : 2), warm in a water-bath for 2 minutes, cool and filter. To the filtrate, add 0.1 mL of sodium nitrate TS and allow to stand in a dark place for 20 minutes. Add 1.5 mL of ammonia solution (28): a yellowish red color is observed.

(2) To a portion of powdered Levothyroxine Sodium Tablets, equivalent to 1 mg of Levothyroxine Sodium according to the labeled amount, add 10 mL of ethanol (95), shake, filter and use the filtrate as the test solution. Dissolve 10 mg of Levothyroxine Sodium RS in 100 mL of ethanol (95) and use this solution as the

standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 20 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of *t*-butyl alcohol, *t*-amyl alcohol, water, ammonia solution (28) and 2-butanone (59 : 32 : 17 : 15 : 7) to a distance of about 12 cm and air-dry the plate. Spray a solution of 0.3 g of ninhydrin in 100 mL of a mixture of 1-butanol and acetic acid (100) (97 : 3) on the plate and heat at 100 °C for 3 minutes: the spots obtained from the test solution and the standard solution show a red-purple color and has the same R_f value.

Purity ***Soluble halides***—Weigh a portion of powdered Levothyroxine Sodium Tablets, equivalent to 2.5 mg of Levothyroxine Sodium according to the labeled amount, add 25 mL of water, warm to 40 °C, shake for 5 minutes, add 3 drops of dilute nitric acid and filter. To the filtrate, add 3 drops of silver nitrate TS and mix: the solution has no more opalescence than the following control solution.

Control solution—To 0.25 mL of 0.01 mol/L hydrochloride VS, add 25 mL of water and 3 drops of dilute nitric acid and proceed as directed above.

Dissolution Test Perform the test with 1 tablet of Levothyroxine Sodium Tablets at 50 revolutions per minute according to Method 2 under Dissolution Test, using 500 mL of 0.01 mol/L hydrochloric acid TS containing 0.2 % sodium lauryl sulfate as the dissolution solution. Take the dissolved solution after 45 minutes from the start of the test and filter. Discard the first 10 mL of the filtrate and use the subsequent filtrate as the test solution. Seperately, weigh accurately a suitable amount of Levothyroxine RS, dissolve in methanol to make a solution containing about 0.1 mg per mL, dilute with the dissolution solution to make the same concentration as the test solution, and use this solution as the standard solution. Perform the test with 800 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine the peak areas, A_T and A_S, of levothyroxine. The dissolution rate of Levothyroxine Sodium Tablets in 45 minutes is not less than 70 % (Q).

Dissolution rate (%) with respect to the labeled amount of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$)

$$= C_S \times \frac{A_T}{A_S} \times \frac{1}{C} \times 50000$$

C_S: Concentration (mg/mL) of the standard solution

C: Labeled amount (mg) of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$) in 1 tablet

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 225 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (1.5 μm to 10 μm in particle diameter).

Mobile phase: A mixture of methanol and 0.1 % phosphoric acid (60:40)

Flow rate: 2 mL/minute

System suitability

System performance: When the procedure is run with 800 μL of the standard solution under the above operating conditions, the symmetry factor is not more than 1.5.

System repeatability: When the test is repeated 5 times with 800 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of levothyroxine is not more than 4.0 %.

Uniformity of Dosage Units It meets the requirement when the content uniformity test is performed according to the following procedure. Place 1 tablet of Levothyroxine Sodium Tablets in a glass-stoppered centrifuge tube, add exactly 10 mL of 0.01 mol/L sodium hydroxide TS, warm at 50 °C for 15 minutes and shake vigorously for 20 minutes. Centrifuge this solution, pipet 5.0 mL of the clear supernatant liquid, add 1.0 mL of the internal standard solution and use this solution as the test solution. Perform the test with 20 μL of the test solution as directed under Liquid Chromatography according to the following conditions and calculate the ratio of the peak area of levothyroxine sodium to that of the internal standard.

Internal standard solution—A solution of Ethinylestradiol in a mixture of acetonitrile and diluted phosphoric acid (1 in 10) (9 : 1) (3 in 40000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: a constant wavelength between 220 nm and 230 nm).

Column: A stainless steel column, 4 mm to 6 mm in internal diameter and 10 cm to 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: A mixture of methanol, water and phosphoric acid (6700 : 3300 : 5).

Flow rate: Adjust the flow rate so that the retention time of levothyroxine sodium is about 9 minutes.

System suitability

System performance: To 5 mL of a solution of Levothyroxine Sodium in 0.01 mol/L sodium hydroxide TS (1 in 200000) and add 1 mL of the internal standard solution. When the procedure is run with 20 μL of this solution under the above operating conditions, levothyroxine sodium and the internal standard

are eluted in this order with a resolution between their peaks being not less than 2.0.

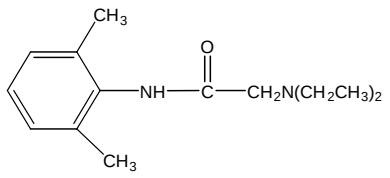
Assay Weigh accurately and powder not less than 20 Levothyroxine Sodium Tablets. Weigh accurately a portion of the powder, equivalent to about 3 mg of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$) into a crucible and add potassium carbonate amounting to twice the weight of the powder. In the case that the weighed powder is less than 4 g, add 8 g of potassium carbonate to the crucible. Mix well and gently tap the crucible on the bench to compact the mixture. Overlay with 10 g of potassium carbonate and compact again by tapping. Heat the crucible strongly at a temperature between 675 °C and 700 °C for 25 minutes. Cool, add 30 mL of water, heat gently to boiling and filter into a flask. To the residue, add 30 mL of water, boil and filter into the same flask. Rinse the crucible and the char on the funnel with hot water until the filtrate measures 300 mL. Add slowly 7 mL of freshly prepared bromine TS and diluted phosphoric acid (1 in 2) in the ratio of 3.5 mL to 1 g of the added potassium carbonate and boil until starch-potassium iodide paper is no longer colored blue by the evolved gas. Wash the inside of the flask with water and continue boiling for 5 minutes. During the boiling, add water from time to time to maintain a volume of not less than 250 mL. Cool, add 5 mL of a solution of phenol (1 in 20), rinse again the inside of the flask with water and allow to stand for 5 minutes. Add 2 mL of diluted phosphoric acid (1 in 2) and 5 mL of potassium iodide TS and titrate immediately the liberated iodine with 0.01 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination and make any necessary correction.

Each mL of 0.01 mol/L sodium thiosulfate VS
= 0.33286 mg of $C_{15}H_{10}I_9NNaO_4$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Lidocaine



$C_{14}H_{22}N_2O$: 234.34

2-(Diethylamino)-*N*-(2,6-dimethylphenyl)acetamide
[*137-58-6*]

Lidocaine, when dried, contains not less than 99.0 % and not more than 101.0 % of lidocaine ($C_{14}H_{22}N_2O$).

Description Lidocaine appears as white to pale yellow crystals or crystalline powder.
Lidocaine is very soluble in methanol or in ethanol (95), soluble in acetic acid (100) or in ether and practically insoluble in water.
Lidocaine dissolves in dilute hydrochloric acid.

Identification (1) Dissolve 40 mg each of Lidocaine and Lidocaine RS in 10 mL each of 1 mol/L hydrochloric acid TS and add water to make 100 mL each. Determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Lidocaine and Lidocaine RS, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 66 ~ 69 °C

Purity (1) ***Clarity and color of solution***— Dissolve 1.0 g of Lidocaine in 2 mL of dilute hydrochloric acid and add water to make 10 mL: the solution is clear and colorless to pale yellow.

(2) ***Chloride***—Dissolve 0.6 g of Lidocaine in 6 mL of dilute nitric acid, add water to make 50 mL and perform the test. Prepare the control solution with 0.70 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.041 %).

(3) ***Sulfate***—Dissolve 0.5 g of Lidocaine in 5 mL of dilute hydrochloric acid, add water to make 50 mL and perform the test. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS, 5 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.096 %).

(4) ***Heavy metals***—Carbonize 2.0 g of Lidocaine by gentle ignition. After cooling, add 10 mL of a solution of magnesium nitrate in ethanol (95) (1 in 10) and burn the ethanol. After cooling, add 1 mL of sulfuric acid, proceed according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(5) ***Related substances***—Dissolve 0.10 g of Lidocaine in 2 mL of methanol and use this solution as the test solution. Pipet 1.0 mL of the test solution, add methanol to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethylacetate, 2-butanone, water and formic acid (5 : 3 : 1 : 1) to a distance of about 10 cm and air-dry the plate and dry at 80 °C for 30 minutes. After cooling, examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test

solution are not more intense than the spot from the standard solution.

(6) ***2,6-Dimethylaniline***—Weigh accurately about 50.0 mg of Lidocaine, dissolve in the mobile phase to make exactly 10.0 mL and use this solution as the test solution. Separately, weigh accurately about 50.0 mg of 2,6-dimethylaniline, dissolve in the mobile phase to make exactly 100.0 mL, pipet 1.0 mL of this solution and add the mobile phase to make exactly 100.0 mL. Pipet 1.0 mL of this solution, add the mobile phase to make exactly 100.0 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions: the amount of 2,6-dimethylaniline having the relative retention time of about 0.40 with respect to lidocaine is not more than 100 ppm.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm)

Column: A stainless steel column about 3.9 mm in internal diameter and about 15 cm in length, packed with octadecylsilyl amorphous organosilica polymer (5 μm in particle diameter).

Column temperature: A constant temperature of about 30 °C

Mobile phase: A mixture of phosphate buffer (pH 8.0) and acetonitrile (70:30)

Flow rate: 1.0 mL/minute

Time span of measurement: About 3.5 times as long as the retention time of lidocaine, beginning after the solvent peak

Phosphate buffer (pH 8.0)—Dissolve 4.85 g of potassium dihydrogen phosphate in 1000 mL of water and adjust the pH to 8.0 with sodium hydroxide solution.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, silica gel, 24 hours).

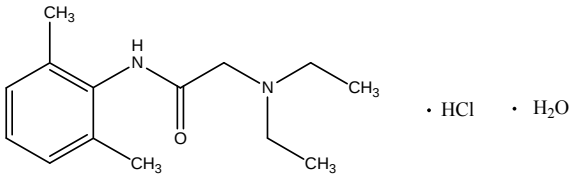
Residue on Ignition Not more than 0.1 % (1 g).

Assay Dissolve 0.5 g of Lidocaine, previously dried and accurately weighed, in 20 mL of glacial acetic acid and titrate with 0.1 mol/L perchloric acid VS (indicator: 1 drop of methylrosaniline chloride TS) until the color of the solution changes from purple through blue to blue-green. Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 23.434 mg of $C_{14}H_{22}N_2O$

Containers and Storage ***Containers***—Tight containers.

Lidocaine Hydrochloride Hydrate



$C_{14}H_{22}N_2O \cdot HCl \cdot H_2O$: 288.81

2-(Diethylamino)-*N*-(2,6-dimethylphenyl)acetamide hydrate hydrochloride [*6108-05-0*]

Lidocaine Hydrochloride Hydrate contains not less than 97.5 % and not more than 102.5 % of lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$), calculated on the anhydrous basis.

Description Lidocaine Hydrochloride Hydrate is a white, crystalline powder, is ordorless and has a slightly bitter taste.

Lidocaine Hydrochloride Hydrate is very soluble in water or in ethanol (95), soluble in chloroform, and practically insoluble in ether.

Identification (1) Dissolve about 0.3 g in 5 to 10 mL of water in a separator, add 4 mL of 6 mol/L ammonium hydroxide TS, and extract with four 15-mL portions of chloroform. Combine the chloroform extracts, evaporate chloroform, and dry the residue in vacuum over silica gel for 24 hours. Determine the infrared spectra of the crystalline precipitate so obtained and Lidocaine Hydrochloride Hydrate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) When proceed as directed in the Assay, the retention time of the principal peak from the test solution corresponds to that from the standard solution.

(3) A solution of Lidocaine Hydrochloride Hydrate responds to the Qualitative Tests (2) for chloride.

Melting Point 74 ~ 79 °C.

Purity (1) ***Sulfate***—Dissolve 0.1 g of Lidocaine Hydrochloride Hydrate in 10 mL of water and use this solution as the test solution. Add 0.10 mL of 0.020 mol/L sulfuric acid to 10 mL of water and use this solution as the control solution. To each of the test solution and the control solution, add 1 mL of 3 mol/L hydrochloric acid and 1 mL of barium chloride TS: the test solution is not more turbid than the control solution (not more than 0.1 %).

(2) ***Heavy metals***—Proceed with 1.0 g of Lidocaine Hydrochloride Hydrate according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

Water 5.0 ~ 7.0 % (0.2 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 %.

Assay Weigh accurately about 0.1 g of Lidocaine Hydrochloride Hydrate, dissolve in the mobile phase to make 50 mL, and use this solution as the test solution. Separately, dissolve about 85 mg of Lidocaine RS in 0.5 mL of 1 mol/L hydrochloric acid, with warming if necessary, dilute with the mobile phase to 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine the peak area (A_T) of lidocaine hydrochloride from the test solution and that (A_S) from the standard solution.

$$\text{Amount (mg) of lidocaine hydrochloride } (C_{14}H_{22}N_2O\cdot HCl) = \text{Amount (mg) of Lidocaine RS} \times \frac{A_T}{A_S} \times \frac{270.80}{234.34}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel 3.9 mm internal diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: 20 ~ 25 °C.

Mobile phase: Mix 50 mL of glacial acetic acid and 930 mL of water, and adjust with 1 mol/L sodium hydroxide to a pH of 3.40. Mix about 4 parts of this solution with 1 part of acetonitrile, so that the retention time of lidocaine is about 4 to 6 minutes.

Flow rate: 1.5 mL/min.

System suitability

System performance: Dissolve 22 mg of methyl parahydroxybenzoate in the mobile phase. Mix 2 mL of this solution and 20 mL of the standard solution. When the procedure is run with 20 μL of this solution, the resolution between the peaks of lidocaine and methyl parahydroxybenzoate is not less than 3.0.

System repeatability: When the test is repeated 5 times with 20 μL each of the standard solution according to the above conditions, the relative standard deviation of the peak area of lidocaine is not more than 1.5 %.

Containers and Storage ***Containers***—Well-closed containers.

Lidocaine Injection

Lidocaine Injection is an aqueous solution for injection. Lidocaine Injection contains not less than 95.0 % and not more than 105.0 % of the labeled amount of lidocaine hydrochloride ($C_{14}H_{22}N_2O\cdot HCl$: 270.80).

Method of Preparation Prepare as directed under Injections, with Lidocaine and an equivalent amount of hydrochloric acid.

No preservative is added in the case of intravenous injections.

Description Lidocaine Injection is a colorless, clear liquid.

pH—5.0 ~ 7.0.

Identification To a volume of Lidocaine Injection, equivalent to 20 mg of lidocaine hydrochloride ($C_{14}H_{22}N_2O\cdot HCl$) according to the labeled amount, add 1 mL of sodium hydroxide TS and extract with 20 mL of hexane. To 10 mL of the hexane extract, add 20 mL of 1 mol/L hydrochloric acid TS and shake vigorously. Determine the absorption spectrum of the water layer as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 261 nm and 265 nm.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 1.1 EU/mg of lidocaine hydrochloride.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay Take an exactly measured volume of Lidocaine Injection, equivalent to about 0.1 g of lidocaine hydrochloride ($C_{14}H_{22}N_2O\cdot HCl$), add 10.0 mL of the internal standard solution and 0.001 mol/L hydrochloric acid TS to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately about 85 mg of Lidocaine RS, previously dried in a desiccator (in vacuum, silica gel) for 24 hours, dissolve in 0.5 mL of 1 mol/L hydrochloric acid TS and a suitable volume of 0.001 mol/L hydrochloric acid TS and add 10.0 mL of the internal standard solution. Then add 0.001 mol/L hydrochloric acid TS to make exactly 50 mL and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of lidocaine to that of the internal standard for the test solution and the standard solution, respectively.

Amount (mg) of lidocaine hydrochloride

$(C_{14}H_{22}N_2O \cdot HCl)$ = Amount (mg) of Lidocaine RS

$$\times \frac{Q_T}{Q_S} \times 1.1556$$

Internal standard solution—A solution of benzophenone in methanol (1 in 4000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature at about 25 °C.

Mobile phase: Dissolve 2.88 g of sodium lauryl sulfate in 1000 mL of a mixture of 0.02 mol/L phosphate buffer solution, pH 3.0 and acetonitrile (11 : 9).

Flow rate: Adjust the flow rate so that the retention time of lidocaine is about 6 minutes.

System suitability

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, lidocaine and the internal standard are eluted in this order with the resolution between their peaks being not less than 6.0.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of peak area of lidocaine to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Lincomycin Hydrochloride Capsules

Lincomycin Hydrochloride Capsules contain not less than 90.0 % and not more than 120.0 % of the labeled amount of lincomycin ($C_{18}H_{34}N_2O_6S$: 406.54).

Method of Preparation Prepare as directed under Capsules, with Lincomycin Hydrochloride Hydrate.

Identification (1) Weigh an amount of the contents of Lincomycin Hydrochloride Capsules, equivalent to 50 mg (potency) of lincomycin hydrochloride, and 50 mg (potency) of Lincomycin Hydrochloride RS, dissolve each in 10 mL of methanol and use these solutions as the test solution and the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-butanone, acetone and water (8:4:1) to a distance of about 10 cm and air-dry the plate. Spray 0.5 % potassium permanganate solution, allow to stand for 10 minutes then spray 0.2 % bromophenol blue solution: the blue spots obtained from the test solution and the standard solution show the same R_f value.

(2) The ratio of the retention time of lincomycin to the retention time of the internal standard in the chromatogram of the test solution corresponds to that in the chromatogram of the standard solution, as obtained in the Assay.

Water Not more than 7.0 % (0.2 g, volumetric titration, direct titration).

Dissolution Test Perform the test with 1 capsule of Lincomycin Hydrochloride Capsules at 100 revolutions per minute according to Method 1, using 500 mL of water as the dissolution solution. Take the dissolved solution after 45 minutes from the start of the test and filter. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution solution to make exactly V' mL and use this solution as the test solution. Separately, weigh accurately a suitable amount of Lincomycin RS, dissolve in the dissolution solution to make the same concentration as the test solution and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed in the Assay under Lincomycin Hydrochloride Hydrate. The dissolution rate of Lincomycin Hydrochloride Capsules in 45 minutes is not less than 75 % (Q).

Dissolution rate (%) with respect to the labeled amount of lincomycin ($C_{18}H_{34}N_2O_6S$)

$$= C_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 50000$$

C_S: Concentration [mg (potency)/mL] of the standard solution

C: Labeled amount [mg (potency)] of lincomycin ($C_{18}H_{34}N_2O_6S$) in 1 capsule

Uniformity of Dosage Units It meets the requirement.

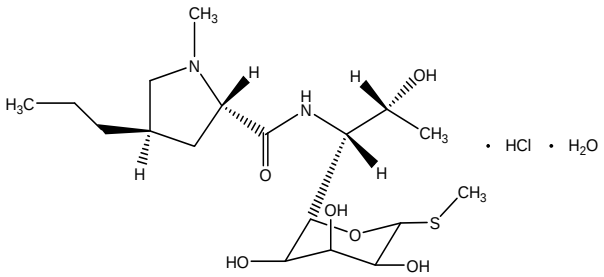
Assay Take exactly an amount of Lincomycin Hydrochloride Capsules, equivalent to about 0.1 g (potency) according to the labeled potency, add the mobile phase to make exactly 100 mL and perform the test as directed in the Assay under Lincomycin Hydrochloride Hydrate.

Amount [μg (potency)] of lincomycin ($C_{18}H_{34}N_2O_6S$) = Amount [μg (potency)] of

Lincomycin Hydrochloride RS $\times \frac{A_T}{A_S} \times 10$

Containers and Storage ***Containers*—**Tight containers.

Lincomycin Hydrochloride Hydrate



$C_{18}H_{34}N_2O_6S \cdot HCl \cdot H_2O$: 461.01

(2*S*,4*R*)-*N*-[(1*R*,2*R*)-2-Hydroxy-1-[(2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-methylsulfanyloxan-2-yl]propyl]-1-methyl-4-propylpyrrolidine-2-carboxamide hydrate hydrochloride [*7179-49-9*]

Lincomycin Hydrochloride Hydrate is the hydrochloride of a substance having antibacterial activity produced by the growth of *Streptomyces lincolnensis* var. *lincolnensis*.
Lincomycin Hydrochloride Hydrate contains not less than 825 μg (potency) per mg of lincomycin ($C_{18}H_{34}N_2O_6S$: 406.54), calculated on the anhydrous basis

Description Lincomycin Hydrochloride Hydrate appears as white crystals or crystalline powder.
Lincomycin Hydrochloride Hydrate is freely soluble in water or in methanol, sparingly soluble in ethanol (95) and very slightly soluble in acetonitrile.

Identification (1) Determine the infrared spectra of Lincomycin Hydrochloride Hydrate and Lincomycin Hydrochloride Hydrate RS, as directed in the paste method under Infrared spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) A solution of Lincomycin Hydrochloride Hydrate (1 in 100) responds to the Qualitative Tests (2) for chloride.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{25}$: +135 ~ +150° (0.5 g, water, 25 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.1 g of Lincomycin Hydrochloride Hydrate in 1 mL of water is between 3.0 and 5.5.

Purity (1) ***Clarity and color of solution*—**Dissolve 1.0 g of Lincomycin Hydrochloride Hydrate in 10 m L of water: the solution is clear and colorless.

(2) ***Heavy metals*—**Proceed with 2.0 g of Lincomycin Hydrochloride Hydrate according to Method 4 and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 5 ppm).

(3) ***Lincomycin B*—**Perform the test with 20 μL of the test solution obtained in the Assay as directed under Liquid Chromatography according to the following operating conditions and determine the peak areas of lincomycin and lincomycin B having the relative retention time of about 0.5 with respect to lincomycin: the peak area of lincomycin B is not more than 5.0 % of the sum of the peak areas of lincomycin and lincomycin B.

Operating conditions

Proceed as directed in the operating conditions in the Assay.

System suitability

System performance and system repeatability: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Pipet 1 mL of the test solution and add the mobile phase to make exactly 20 mL. Confirm that the peak area of lincomycin obtained from 20 μL of this solution is equivalent to 3.5 % to 6.5 % of that from 20 μL of the standard solution.

Water 3.0 ~ 6.0 % (0.5 g, volumetric titration, direct titration).

Sterility Test It meets the requirement, when Lincomycin Hydrochloride Hydrate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.5 EU/mg (potency) of lincomycin hydrochloride, when Lincomycin Hydrochloride Hydrate is used in a sterile preparation.

Histamine It meets the requirement, When Lincomycin Hydrochloride Hydrate is used in a sterile preparation. Weigh appropriate amount of Lincomycin Hydrochloride Hydrate, dissolve in Isotonic sodium chloride Injection solution, make the solution so that each mL contains 3.0 mg (potency), and use the solution as the test solution.

Assay Weigh accurately about 10 mg (potency) each of Lincomycin Hydrochloride Hydrate and Lincomycin Hydrochloride Hydrate RS, dissolve each in a suitable amount of the mobile phase, add the mobile phase to make exactly 10 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 20 μL of each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine the peak areas, A_T and A_S, of

lincomycin.

$$\text{Amount [μg (potency)] of lincomycin } (C_{18}H_{34}N_2O_6S) = \text{Amount [μg (potency)] of Lincomycin Hydrochloride RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm)

Column: A stainless steel column about 4 mm in internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 46 °C

Mobile phase: To 13.5 mL of phosphoric acid, add water to make 1000 mL and adjust the pH to 6.0 with ammonia TS. To 780 mL of this solution, add 150 mL of acetonitrile and 150 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of lincomycin is about 9 minutes.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of lincomycin is not less than 4000 with the symmetry factor being not more than 1.3.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of lincomycin is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Lincomycin Hydrochloride Injection

Lincomycin Hydrochloride Injection is an aqueous solution for injection. Lincomycin Hydrochloride Injection contains not less than 93.0 % and not more than 107.0 % of lincomycin ($C_{18}H_{34}N_2O_6S$: 406.54).

Method of Preparation Prepare as directed under Injections, with Lincomycin Hydrochloride Hydrate.

Description Lincomycin Hydrochloride Injection is a clear, colorless liquid.

Identification To a volume of Lincomycin Hydrochloride Injection, equivalent to 30 mg (potency) of Lincomycin Hydrochloride Hydrate according to the labeled amount, add 30 mL of water and use this solution as the test solution. Separately, dissolve 10 mg (potency) of Lincomycin Hydrochloride RS in 10 mL of water and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Dissolve 150 g of ammonium acetate in 800 mL of water, adjust the pH to 9.6 with ammonia solution (28) and add water to make 1000 mL. To 80 mL of this solution, add 40 mL of 2-propanol and 90 mL of ethyl acetate and shake. Develop the plate with the upper layer of this solution to a distance of about 15 cm and air-dry the plate. Spray evenly a solution of potassium permanganate (1 in 1000): the principal spots from the test solution and the spots from the standard solution show the same R_f value.

pH 3.0 ~ 5.5.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.5 EU/mg (potency) of lincomycin.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

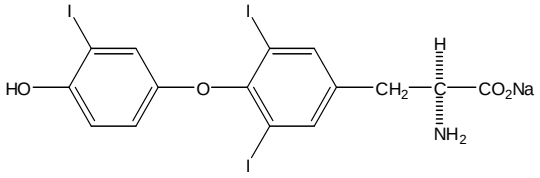
Determination of Volume of Injection in Containers It meets the requirement.

Assay Pipet a volume of Lincomycin Hydrochloride Injection, equivalent to about 0.1 g (potency) according to the labeled potency, add the mobile phase to make exactly 100 mL and proceed as directed in the Assay under Lincomycin Hydrochloride Hydrate.

$$\text{Amount [μg (potency)] of lincomycin } (C_{18}H_{34}N_2O_6S) = \text{Amount [μg (potency)] of Lincomycin Hydrochloride RS} \times \frac{A_T}{A_S} \times 10$$

Containers and Storage ***Containers*—**Hermetic containers.

Liothyronine Sodium



$C_{15}H_{11}I_3NNaO_4$: 672.96

Sodium (2*S*)-2-amino-3-[4-(4-hydroxy-3-iodophenoxy)-3,5-diiodophenyl]propanoate [*55-06-1*]

Liothyronine Sodium contains not less than 95.0 % and not more than 101.0 % of liothyronine sodium ($C_{15}H_{11}I_3NNaO_4$), calculated on the dried basis.

Description Liothyronine Sodium is a white to pale brown powder and is odorless.
Liothyronine Sodium is slightly soluble in ethanol (95) and practically insoluble in water or in ether.
Liothyronine Sodium dissolves in sodium hydroxide TS or in ammonia TS.

Identification (1) Take 5 mL of a solution of Liothyronine Sodium in ethanol (95) (1 in 1000), add 1 mL of ninhydrin TS and warm in a water-bath for 5 minutes: a purple color is observed.

(2) Heat 20 mg of Liothryonine Sodium with a few drops of sulfuric acid over a flame: a purple gas is evolved.

(3) Determine the absorption spectra of solutions of Liothyronine Sodium and Liothyronine Sodium RS in ethanol (95) (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Ignite 20 mg of Liothyronine Sodium until thoroughly charred. After cooling, add 5 mL of water to residue, shake and filter: the filtrate responds to the Qualitative Tests (1) for sodium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: +18 ~ +22° (0.2 g, calculated on the dried basis, a mixture of ethanol (95) and 1 mol/L hydrochloric acid TS (4 : 1), 10 mL, 100 mm).

Purity (1) ***Chloride***—Transfer 0.1 g of Liothyronine Sodium, previously dried, to a platinum dish and incinerate, protecting the dish from air currents during the incineration. When carbonization is complete, cool, moisten with 2 drops of water and break up the mass with a glass rod. Add 10 mL of water and 5 mL of ammonia solution (28), mix and transfer to a 50 mL flask. Wash the platinum dish with water and add the washings to the flask to make a total volume of 25 mL. Add 10 mL of silver chloride solution (1 in 20), shake, filter and transfer to a Nessler tube. Wash the flask and filter paper with 10 mL of water and add the washings to the tube. Acidify the combined filtrate and washings with nitric acid and dilute with 50 mL of water. Separately, mix 5 mL of ammonia solution (28), 20 mL of water and 10 mL of silver nitrate solution (1 in 20), filter and transfer to a Nessler tube. Wash the filter paper with 10 mL of water and add the washings to the tube. Acidify the combined filtrate and washings with nitric acid, dilute with water to make 50 mL and use this solution as the control solution. Add sodium chloride solution (1in 1000) to the control solution until the turbidity of the control solution matches that of the test solution: not more than 2.0 mL is consumed (not more than 1.2 %).

(2) ***Soluble halide***—Take 10 mg of Liothyronine Sodium, add 10 mL of water and 1 drop of dilute nitric acid, shake for 5 minutes and filter. Add water to the filtrate to make 10 mL and mix with 3 drops of silver nitrate TS: the solution shows no more turbidity than the following control solution.

Control solution—To 0.35 mL of 0.01 mol/L hydrochloric acid VS, add 1 drop of dilute nitric acid and water to make 10 mL and add 3 drops of silver nitrate TS.

(3) ***Iodine and iodide***—Dissolve 0.1 g of Liothryonine Sodium in 10 mL of dilute sodium hydroxide TS and 15 mL of water, add 5 mL of dilute sulfuric acid and allow to stand for 10 minutes with occasional shaking. Filter the mixture into a Nessler tube, add 10 mL of chloroform and 3 drops of a solution of potassium iodide (1 in 100) to the filtrate, mix for 30 seconds and allow to stand: the chloroform layer has no more color than the following control solution.

Control solution—Weigh exactly about 0.111g of potassium iodide and dissolve in water to make 1000 mL. Pipet 1.0 mL of this solution, add 10 mL of dilute sodium hydroxide TS, 14 mL of water and 5 mL of dilute sulfuric acid and mix. Filter the mixture into a Nessler tube and perform the test with the filtrate in the same manner as for the sample.

(4) ***Related substances***—Dissolve 0.15 g of Liothyronine Sodium in 5 mL of diluted ammonia TS (1 in 3) and use this solution as the test solution. Pipet 1 mL of this solution, add diluted ammonia TS (1 in 3) to make exactly 50 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 1 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of *t*-butyl alcohol, *t*-amyl alcohol, water, ammonia solution (28) and 2-butanone (59 : 32 : 17 : 15 : 7) to a distance of about 12 cm and air-dry the plate. Spray evenly solution of 0.3 g of ninhydrin in 100 mL of a mixture 1-butanol and acetic acid (100) (97 : 3) on the plate and dry the plate at 100 °C for 3 minutes: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 4.0 % (0.2 g, 105 °C, 2 hours).

Assay Weigh accurately about 25 mg of Liothyronine Sodium and proceed as directed under the Oxygen Flask Combustion Method, using a mixture of 10 mL of a solution of sodium hydroxide (1 in 100) and 1 mL of a freshly prepared solution of sodium bisulfate (1 in 100) as the absorbing liquid and prepare the test solution. Apply a small amount of water to the upper part of apparatus A, pull out C carefully and wash C, B and

the inner wall of A with 40 mL of water. To the test solution, add 1 mL of bromine-acetic acid TS, insert the stopper C and shake vigorously for 1 minute. Wash C, B and the inner wall of A with 40 mL of water and add 0.5 mL of formic acid. Stopper the flask with C and shake vigorously for 1 minute again. Wash C, B and the inner wall of A with 40 mL of water again. Bubble the solution with enough nitrogen gas in the flask to remove the oxygen and excess bromine, add 0.5 g of potassium iodide to the solution and dissolve. Add immediately 3 mL of dilute sulfuric acid, mix and allow to stand for 2 minutes. Titrate the solution with 0.02 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination and make any necessary correction.

Each mL of 0.02 mol/L sodium thiosulfate VS
= 0.7477 mg of $C_{15}H_{11}I_3NNaO_4$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Liothyronine Sodium Tablets

Liothyronine Sodium Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of liothyronine sodium ($C_{15}H_{11}I_3NNaO_4$: 672.96).

Method of Preparation Prepare as directed under Tablets, with Liothyronine Sodium.

Identification (1) To a glass-stoppered centrifuge tube, add a portion of finely powdered Liothyronine Sodium Tablets, equivalent to 0.1 mg of Liothyronine Sodium according to the labeled amount, add 30 mL of dilute sodium hydroxide TS, shake vigorously and centrifuge. Transfer the clear supernatant liquid to a separatory funnel, add 10 mL of dilute hydrochloric acid and extract with two 20 mL volumes of ethyl acetate. Filter each extract successively through absorbent cotton, previously overlaid with 8 g of anhydrous sodium sulfate. Evaporate the filtrate on a water-bath to dryness with the aid of a current of nitrogen. Dissolve the residue in 0.5 mL of methanol and use this solution as the test solution. Separately, dissolve 10 mg of Liothyronine Sodium RS in methanol to make 50 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 20 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of *t*-butyl alcohol, *t*-amyl alcohol, water, ammonia solution (28) and 2-butanone (59 : 32 : 17 : 15 : 7) to a distance of about 12 cm and air-dry the plate. Spray evenly a solution of 0.3 g of ninhydrin in 100 mL of a mixture of 1-butanol and acetic acid (100) (97 : 3) on the plate and dry the plate at 100 °C for 3 minutes: the spots obtained from the test solution and the standard solution show a red-purple color and has the same R_f value.

(2) The colored solution obtained in the Assay is blue in color.

Disintegration Test It meets the requirement.

Uniformity of Dosage Units It meets the requirement when the content uniformity test is performed according to the following method.

Place 1 tablet of Liothyronine Sodium Tablets in a glass-stoppered centrifuge tube and exactly 10 mL of 0.01 mol/L sodium hydroxide TS, warm at 50 °C for 15 minutes and shake vigorously for 20 minutes. Centrifuge for 5 minutes and filter the clear supernatant liquid, if necessary. Pipet a definite volume of this solution and add a volume of 0.01 mol/L sodium hydroxide VS to prepare a definite volume of a solution containing about 0.5 μg of liothyronine sodium ($C_{15}H_{11}I_3NNaO_4$) per mL. Pipet 5 mL of this solution, add 1 mL of the internal standard solution and use this solution as the test solution. Perform the test with 200 μL of the test solution as directed under Liquid Chromatography according to the following conditions and calculate the ratio of the peak area of the Liothyronine to that of the internal standard.

Internal standard solution—A solution of propylparahydroxybenzoate in a mixture of methanol and diluted phosphoric acid (1 in 10) (9 : 1) (1 in 250000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column, 4.6 mm in internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: A mixture of methanol and water (57 : 43).

Flow rate: Adjust the flow rate so that the retention time of liothyronine is about 9 minutes.

System suitability

System performance: Take 5 mL of a solution of Liothyronine Sodium in 0.01 mol/L sodium hydroxide TS (1 in 2000000) and add 1 mL of the internal standard solution. When the procedure is run with 200 μL of this solution under the above operating conditions, internal standard and liothyronine are eluted in this order with a resolution between their peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 200 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of

liothyronine to that of the internal standard is not more than 1.0 %.

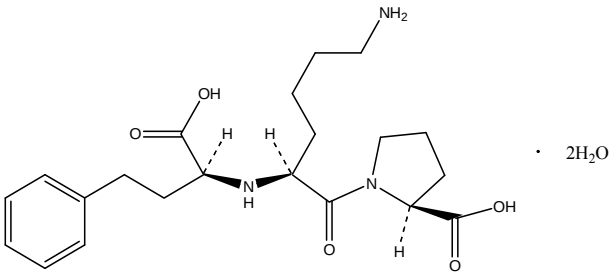
Assay Weigh accurately not less than 20 Liothyronine Sodium Tablets and finely powder. Place a portion of the powder accurately weighed, equivalent to about 50 mg of liothyronine sodium ($C_{15}H_{11}I_3NNaO_4$), in an agate mortar and add 1 g of powdered potassium carbonate and mix well. Transfer the mixture cautiously to a porcelain crucible and compact the contents by gently tapping the crucible on a table. Add an additional 1.5 g of powdered potassium carbonate to the same agate mortar, mix well with any content adhering to the mortar, cautiously overlay the mixture on the top of the same porcelain crucible and compact the contents again in the same manner. Ignite the combined mixture in the crucible between 675 °C and 700 °C for 30 minutes. Cool, add a few mL of water to the crucible, heat gently to boil and filter the contents of the crucible through a glass filter (G4) into a volumetric flask. Wash the residue with water and combine the washings with the filtrate. Cool, add water to make 20 mL and use this solution as the test solution. Separately, weigh accurately about 75 mg of Potassium Iodide RS, previously dried at 105 °C for 4 hours and dissolve in water to make exactly 200 mL. Measure exactly 5 mL of the solution and add a solution of potassium carbonate (1 in 8) to make exactly 100 mL. Take 2 mL of this solution, add a solution of potassium carbonate (1 in 8) to make exactly 20 mL and use this solution as the standard solution. Pipet 5 mL each of the test solution and the standard solution into glass-stoppered test tubes, add 3.0 mL of diluted sulfuric acid (4 in 25) and 2.0 mL of potassium permanganate TS and heat in a water-bath for 15 minutes. Cool, add 1.0 mL of diluted sodium nitrite TS (1 in 10), swirl to mix, and add 1.0 mL of a solution of ammonium amidosulfate (1 in 10). Allow to stand at room temperature for 10 minutes. Then add 1.0 mL of potato starch TS and 1.0 mL of a freshly prepared diluted potassium iodide TS (1 in 40), swirl to mix and transfer each solution to a volumetric flask. Rinse the test tube with water, collect the washings in the volumetric flask, add water to make 20 mL and allow to stand for 10 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry, using a solution prepared with 5 mL of potassium carbonate (1 in 8) in the same manner as the test solution as the blank. Determine the absorbances, A_T and A_S, of the test solution and the standard solution at the wavelength of a maximum absorption at about 600 nm, respectively.

$$\text{Amount (mg) of lithothyronine sodium } (C_{15}H_{11}I_3NNaO_4) = \text{Amount (mg) of Potassium Iodide RS} \times \frac{A_T}{A_S} \times \frac{1}{2000} \times 1.3513$$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Lisinopril Hydrate



$C_{21}H_{31}N_3O_5 \cdot 2H_2O$: 441.52

(2*S*)-1-[(2*S*)-6-Amino-2-[[(1*S*)-1-carboxy-3-phenylpropyl]amino]hexanoyl]pyrrolidine-2-carboxylic acid dihydrate [*83915-83-7*]

Lisinopril Hydrate contains not less than 98.0 % and not more than 101.0 % of lisinopril ($C_{21}H_{31}N_3O_5$: 405.49), calculated on the anhydrous basis.

Description Lisinopril Hydrate is a white crystalline powder and has a slight, characteristic odor .
It is soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (95).

Melting point—About 160 °C (with decomposition).

Identification (1) Determine the absorption spectra of solutions of Lisinopril Hydrate and Lisinopril Hydrate RS in methanol (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Lisinopril Hydrate and Lisinopril Hydrate RS as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: -43.0 ~ -47.0° (0.25 g calculated on the anhydrous basis, 0.25 mol/L zinc acetate buffer solution, pH 6.4, 25 mL, 100 mm).

0.25 mol/L zinc acetate buffer solution—To 600 mL of water, add 150 mL of acetic acid (100) and 54.9 g of zinc acetate. Mix to dissolve by stirring, add 150 mL of ammonia solution (28) to cool to room temperature. Add ammonia solution (28) to adjust the pH to 6.4 and water to make 1000 mL.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Lisinopril Hydrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Dissolve 0.10 g of Lisinopril Hydrate in 50 mL of water, and use this solution as the test solution. Pipet 3 mL of the test solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 15 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 1.2 with respect to lisinopril, is not larger than 1/5 times the peak area of lisinopril from the standard solution, and the area of the peak other than lisinopril and the peak mentioned above is not larger than 2/15 times the peak area of lisinopril from the standard solution. The total area of related substances other than lisinopril is not larger than the peak area of lisinopril from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column about 4.0 mm in internal diameter and about 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 60 °C.

Mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: Diluted 0.05 mol/L sodium dihydrogen phosphate TS (1 in 2).

Mobile phase B: A mixture of diluted 0.05 mol/L sodium dihydrogen phosphate TS (1 in 2) and acetonitrile for liquid chromatography (3:2).

Time (min)	Mobile phaseA (vol %)	Mobile phaseB (vol %)
0-10	90→50	10→50
10-25	50	50

Flow rate: 1.5 mL per minute.

System suitability

Test for required detectability: Measure exactly 2.5 mL of the standard solution, and add water to make exactly 50 mL. Confirm that the peak area of lisinopril obtained with 15 μL of this solution is equivalent to 3.5 to 6.5 % of that with 15 μL of the standard solution.

System performance: To 10 mg of Lisinopril Hydrate, add 2 mL of a solution of anhydrous caffeine (1 in 1000) and water to make 200 mL. When the procedure is run with 15 μL of this solution under the above operating conditions, lisinopril and caffeine are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 15 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of lisinopril is not more than 2.0 %.

Time span of measurement: About 2.5 times as long as the retention time of lisinopril beginning after the solvent peak.

Water Not less than 8.0 % and not more than 9.5 % (0.3 g, volumetric titration, back titration).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.66 g of Lisinopril Hydrate, dissolve in 80 mL of water, and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 40.55 mg of $C_{21}H_{31}N_3O_5$

Containers and Storage ***Containers***—Well-closed containers.

Lithium Carbonate

Li_2CO_3: 73.89

Dilithium carbonate [*554-13-2*]

Lithium Carbonate, when dried, contains not less than 99.5 % and not more than 101.0 % of lithium carbonate (Li_2CO_3).

Description Lithium Carbonate is a white, crystalline powder and is odorless.

Lithium Carbonate is sparingly soluble in water, slightly soluble in hot water and practically insoluble in ethanol (95) or in ether.

Lithium Carbonate dissolves in dilute acetic acid.

pH—The pH of a solution of Lithium Carbonate (1 in 100) is between 10.9 and 11.5.

Identification (1) Dissolve 0.2 g of Lithium Carbonate in 3 mL of dilute hydrochloric acid and add 4 mL of sodium hydroxide TS and 2 mL of dibasic sodium phosphate TS: a white precipitate is produced. To the precipitate, add 2 mL of hydrochloric acid: it dissolves.

(2) A solution of Lithium Carbonate (1 in 100) responds to the Qualitative Tests for carbonate.

(3) Perform the test as directed under the Flame Coloration Test (1) with Lithium Carbonate: a persistent red color appears.

Purity (1) ***Clarity and color of solution***—Dissolve 0.10 g of Lithium Carbonate in 10 mL of water by warming: the solution is clear and colorless.

(2) ***Acetic acid insoluble substances***—Take 1.0 g of Lithium Carbonate, dissolve in 40 mL of dilute ace-

tic acid, filter the insoluble substances using filter paper for assay, wash with five 10 mL volumes of water and ignite the insoluble substances together with the filter paper to incinerate: the weight of the residue is not more than 1.5 mg.

(3) ***Chloride***—To 0.40 g of Lithium Carbonate, add 10 mL of water and 7 mL of dilute nitric acid and dissolve by heating to boil. After cooling, add 6 mL of dilute nitric acid and dilute with water to make 50 mL. Perform the test. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.022 %).

(4) ***Sulfate***—To 0.40 g of Lithium Carbonate, add 10 mL of water and 4 mL of dilute hydrochloric acid and dissolve by heating to boil. After cooling, add 1 mL of dilute hydrochloric acid and dilute with water to make 50 mL. Perform the test. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048 %).

(5) ***Heavy metals***—To 4.0 g of Lithium Carbonate, add 5 mL of water, gradually add 10 mL of hydrochloric acid while mixing and dissolve. Evaporate the solution on a water-bath to dryness. To the residue, add 10 mL of water and dissolve. Place this solution in a Nessler tube, add 1 drop of phenolphthalein TS, add ammonia TS until the soultion shows a spale red color, then add 2 mL of dilute acetic acid and dilute with water to make 50 mL. Perform the test. Prepare the control solution as follows: evaporate 10 mL of hydrochloric acid on a water-bath to dryness, to this residue, add 10 mL of water and dissolve. Place the solution in a Nessler tube, add 1 drop of phenolphthalein TS, add ammonia TS until the solution shows a pale red color, then add 2.0 mL of standard lead solution and 2 mL of dilute acetic acid and dilute with water to make 50 mL (not more than 5 ppm).

(6) ***Sodium***—Weigh accurately about 0.8 g of Lithium Carbonate, dissolve in water to make exactly 100 mL, and use this solution as the test stock solution. Pipet 25 mL of the test stock solution, add water to make exactly 100 mL, and use this solution as the test solution. Separately, weigh accurately 25.4 mg of sodium chloride, dissolve in water to make exactly 1000 mL, and use this solution as the standard solution. Pipet 25 mL of the test stock solution, add exactly 20 mL of the standard solution, add water to make exactly 100 mL, and use this solution as the standard additive solution. Determine the emission intensities of sodium using a flame photometer with the test solution and standard additive solution under the following conditions. Adjust the wavelength dial to 589 nm, atomize the standard additive solution into the flame, then adjust the sensitivity so that the emission intensity, L_S, shows a reading of about 100, and determine the emission intensity, L_T, of the test solution. Then, change the wavelength to 580 nm, make the other conditions identical, determine the emission intensity, L_B of the test solution, and calculate the amount of sodium by the following equation: not more than 0.05 %.

$$\text{Amount (\%) of sodium (Na)} = \frac{L_T - L_B}{L_S - L_T} \times \frac{W'}{W} \times 100$$

W: Amount (mg) of the sample in the test stock solution

W': Amount (mg) of sodium in 20 mL of the standard solution

(7) ***Magnesium***—To 5.0 g of Lithium Carbonate add 20 mL of water, add slowly 15 mL of hydrochloric acid to dissolve while stirring, and evaporate to dryness in a water bath. Dissolve the residue in 50 mL of water, filter if necessary, and use the filtrate as the solution A. Separately, evaporate 15 mL of hydrochloric acid to dryness in a water bath, proceed in the same manner, and use this solution as the solution B. To 3.0 mL of the solution A add 0.2 mL of a solution of titan yellow (1 in 1000) and water to make 20 mL, add 5 mL of a solution of sodium hydroxide (3 in 20), and allow to stand for 10 minutes: the solution has no more color than the following control solution.

Control solution—Dissolve 49.5 mg of magnesium sulfate heptahydrate, previously dried at 105 °C for 2 hours then heated at 450 °C for 3 hours, in water to make 1000 mL. To 6 mL of this solution add 3 mL of the solution B, 0.2 mL of a solution of titan yellow (1 in 1000), and water to make 20 mL, and proceed in the same manner.

(8) ***Barium***—To 20 mL of the solution A obtained in (7), add 6 mL of water, 0.5 mL of dilute hydrochloric acid, 3 mL of ethano1 (95) and 2 mL of potassium sulfate TS and allow to stand for 1 hour: the solution has no more turbidity than the following control solution.

Control solution—Dissolve 0.0178 g of barium chloride dihydrate in water to make 1000 mL. To 6 mL of this solution, add 20 mL of the solution B obtained in (7), 0.5 mL of dilute hydrochloric acid and 3 mL of ethanol and proceed in the same manner.

(9) ***Aluminum***—To 10 mL of the solution A from (7) add 10 mL of water and 5 mL of pH 4.5 acetic acid-sodium acetate buffer solution, shake, then add 1 mL of a solution of L-ascorbic acid (1 in 100), 2 mL of aluminon TS, and water to make 50 mL, shake well, and allow to stand for 10 minutes: the solution has no more color than the following control solution.

Control solution—Dissolve 0.1758 g of aluminum potassium sulfate hydrate in water to make 1000 mL. To 1.0 mL of this solution add 10 mL of the solution B from (7) and water to make 20 mL, add 5 mL of pH 4.5 acetic acid-sodium acetate buffer solution, and proceed in the same manner.

(10) ***Iron***—Proceed with 1.0 g of Lithium Carbonate according to Method 2, and perform the test according to Method B. Prepare the test solution with 11 mL of dilute hydrochloric acid, and prepare the control solution with 1.0 mL of standard iron solution (not more than 10 ppm).

(11) ***Potassium***—Dissolve 1.0 g of Lithium Carbonate in water to make 100 mL and use this solution as the test solution. To 5 mL of the test solution, add 1.0 mL of dilute acetic acid, shake, add 5 mL of a solution of sodium tetraphenylborate (1 in 30), shake immediately and allow to stand for 10 minutes: the solution has no more turbidity than the following control solution.

Control solution—Dissolve 9.5 mg of potassium chloride in water to make 1000 mL. To 5 mL of this solution, add 1.0 mL of dilute acetic acid, shake and proceed in the same manner.

(12) ***Calcium***—Weigh accurately about 5.0 g of Lithium Carbonate, add 50 mL of water and 15 mL of hydrochloric acid and dissolve. Remove carbon dioxide from the solution by boiling, add 5 mL of ammonium oxalate TS, then make alkaline with ammonia TS and allow to stand for 4 hours. Filter the produced precipitate through a glass filler, wash with warm water until the turbidity of the washing is not produced with calcium chloride TS within 1 minute. Transfer the precipitate and the glass filter into a beaker, add water until the glass filter is covered with water, then add 3 mL of sulfuric acid, heat between 70 °C and 80 °C and titrate with 0.02 mol/L potassium permanganate VS until a pale red color persists for 30 seconds: the amount of calcium (Ca: 40.08) is not more than 0.05 %.

Each mL of 0.02 mol/L potassium permanganate VS
= 2.0039 mg of Ca

(13) ***Arsenic***—Prepare the test solution with 1.0 g of Lithium Carbonate, add 2 mL of water and 3 mL of hydrochloric acid and perform the test (not more than 2 ppm).

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

Assay Weigh accurately about 1.0 g of Lithium Carbonate, previously dried, add 100 mL of water and exactly 50 mL of 0.5 mol/L sulfuric acid VS, remove carbon dioxide by boiling gently, cool and titrate the excess sulfuric acid with 0.5 mol/L sodium hydroxide VS until the color of the solution changes from red to yellow (indicator: 3 drops of methyl red TS). Perform a blank determination and make any necessary correction.

Each mL of 0.5 mol/L sulfuric acid VS
= 36.945 mg of Li_2CO_3

Containers and Storage ***Containers***—Well-closed containers.

Lithium Carbonate Capsules

Lithium Carbonate Capsules contain not less than 95.0 % and not more than 105.0 % of the labelled amount of lithium carbonate (Li_2CO_3: 73.89).

Method of Preparation Prepare as directed under Capsules, with Lithium Carbonate.

Identification A portion of the contents of the capsule responds to the Identification under Lithium Carbonate.

Dissolution Test Perform the test with 1 capsule of Lithium Carbonate Capsules at 100 revolutions per minute according to Method 1 under the Dissolution Test, using 900 mL of water as the dissolution solution. Add water to the dissolved solution to make exactly 1000 mL after 30 minutes from the start of the test and filter. Transfer 20 mL of the filtrates to a volumetric flask, add 500 mL of water, 1 drop of hydrochlroric acid and 20 mL of a suitable surfactant solution and mix. Add water to this solution to make exactly 1000 mL and use this solution as the test solution. Perform the test as directed in the Assay.
The dissolution rate of Lithium Carbonate Capsules in 30 minutes is not less than 80.0 %.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately the content of not less than 20 Lithium Carbonate Capsules. Weigh accurately a portion of the content, equivalent to about 0.6 g of lithium carbonate (Li_2CO_3) in a volumetric flask, add 40 mL of water and 5 mL of hydrochloric acid and shake until the solid is well disintegrated. Dilute with water to make exactly 1000 mL and filter. Pipet 10 mL of the filtrates, add 800 mL of water, 20 mL of the surfactant solution, and water to make exactly 1000 mL, and use this solution as the test solution. Perform the test as directed in the Assay under Lithium Carbonate Tablets.

$$\text{Amount (mg) of lithium carbonate } (Li_2CO_3) = \text{Amount (mg) of Lithium Carbonate RS} \times \frac{P_T}{P_S} \times 20$$

Containers and Storage ***Containers***—Well-closed containers.

Lithium Carbonate Tablets

Lithium Carbonate Tablets contain not less than 95.0 % and not more than 105.0 % of the labeled amount of lithium carbonate (Li_2CO_3: 73.89).

Method of Preparation Prepare as directed under Tablets, with Lithium Carbonate.

Identification A portion of the powered tablets reponds to the Identification under Lithium Carbonate.

Dissolution Test Perform the test with 1 tablet of Lithium Carbonate Tablets at 100 revolutions per minute according to Method 1 under the dissolution test, using 900 mL of water as the dissolution solution. Add water to the dissolved solution to make exactly 1000 mL after 30 minutes of the test and filter. Take exactly 20 mL of the filtrates, add 500 mL of water, 1 drop of hydrochlroric acid and 20 mL of a suitable surfactant solution and mix, add water to make exactly 1000 mL and use this solution as the test solution. Perform the test with the test solution as directed in the Assay.
The dissolution rate of Lithium Carbonate Tablets in 30 minutes is not less than 80 %.

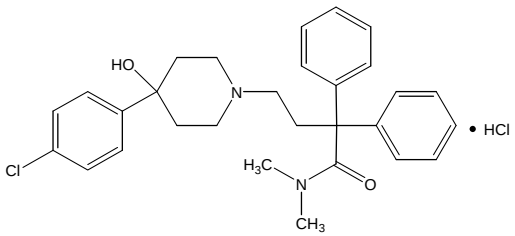
Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Lithium Carbonate Tablets. Weigh accurately a portion of powder, equivalent to about 0.6 g of lithium carbonate (Li_2CO_3), add 40 mL of water and 5 mL of hydrochloric acid and shake until the solid is well disintegrated. Add water to make exactly 1000 mL and filter. Pipet 10 mL of the filtrates, add 800 mL of water and 20 mL of a suitable surfactant solution, add water to make exactly 1000 mL and use this solution as the test solution. Separately, weigh accurately about 30 mg of Lithium Carbonate RS, previously dried at 200 °C for 4 hours, add about 20 mL of water and 0.5 mL of hydrochloric acid, shake to dissolve and add water to make exactly 100 mL. Pipet 20 mL of this solution, add 800 mL of water and 20 mL of a suitable surfactant solution, add water to make exactly 1000 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution using a flame photometer and determine emission intensity, P_T and P_S for the test solution and the standard solution, respectively, at 671 nm.

$$\text{Amount (mg) of lithium carbonate } (Li_2CO_3) = \text{Amount (mg) of Lithium Carbonate RS} \times \frac{P_T}{P_S} \times 20$$

Containers and Storage ***Containers***—Well-closed containers.

Loperamide Hydrochloride



$C_{29}H_{33}ClN_2O_2 \cdot HCl$: 513.50

4-[4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl]-*N,N*-dimethyl-2,2-diphenylbutanamide hydrochloride [*34552-83-5*]

Loperamide Hydrochloride, contains not less than 98.0 % and not more than 102.0 % of loperamide hydrochloride ($C_{29}H_{33}ClN_2O_2 \cdot HCl$), calculated on the dried basis.

Description Loperamide Hydrochloride is a white to pale yellow powder.
Loperamide Hydrochloride is freely soluble in methanol, in 2-propanol or in chloroform and practically insoluble in water or in dilute acid solution.

Melting point—About 225 °C (with decomposition).

Identification (1) Weigh accurately about 40 mg each of Loperamide Hydrochloride and Loperamide Hydrochloride RS, transfer each to 100 mL volumetric flasks, dissolve by the addition of about 50 mL of 2-propanol each, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Loperamide Hydrochloride and Loperamide Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity (1) ***Chloride content***—Weigh accurately about 13 mg of Loperamide Hydrochloride and perform the test as directed under the Oxygen Flask Combustion Method. In particular, use a mixture of 10 mL of 0.02 mol/L sodium hydroxide TS and 2 drops of 30 % hydrogen peroxide TS as the adsorbing liquid of the test. When the combustion is complete, rinse the stopper, sample holder and the inner wall with 50 mL of 2-propanol. Add 4 mL of 0.1 mol/L nitric acid TS and titrate with 0.01 mol/L mercuric nitrate VS using diphenylcarbazone TS as the indicator. The chloride content is not less than 13.52 % and not more than 14.20 %.

Each mL of 0.01 mol/L mercuric nitrate
= 0.3545 mg of Cl

(2) ***Related Substances***—Dissolve Loperamide Hydrochloride and Loperamide Hydrochloride RS in chloroform to make 10 mg per mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of these solutions to a plate of silica gel for thin-layer chromatography and develop the plate using a mixture of chloroform, methanol and formic acid (85 : 10 : 5) to a distance of about 15 cm, air-dry the plate and expose the plate to fumes of iodine: the principal spot obtained from the test solution corresponds in R_f value, color and intensity to that from the standard solution and no other spots are observed.

(3) ***Heavy metals***—Proceed with 1.0 g of Loperamide Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 2.0 ml of standard lead solution (not more than 20 ppm).

Loss on Drying Not more than 0.5 % (1 g, in vacuum drying, 80 °C, 4 hours).

Residue on Ignition Not more than 0.20 % (1 g).

Assay Dissolve about 0.375 g, accurately weighed, of Loperamide Hydrochloride in 25 mL of neutralized acetic acid, add 10 mL of mercuric acetate solution (prepared by dissolving 1 g of mercuric acetate in 33 mL of neutralized acetic acid) and titrate with 0.1 mol/L perchloric acid VS to the original green color.

Each mL of 0.1 mol/L perchloric acid VS
= 51.35 mg of $C_{29}H_{33}ClN_2O_2 \cdot HCl$

Neutralized acetic acid—Dissolve 10 mg of 1–naphtholbenzein in 100 mL of acetic acid (100), titrate with 0.1 mol/L perchloric acid to a green endpoint and use this solution as the neutralized acetic acid. In this preparation, disregard the amount of titrant consumed.

Containers and Storage ***Containers***—Tight containers.

Loperamide Hydrochloride Capsules

Loperamide Hydrochloride Capsules contain not less than 90.0 % and not more than 110.0 % of the labeled amount of loperamide hydrochloride ($C_{29}H_{33}ClN_2O_2 \cdot HCl$: 513.51).

Method of Preparation Prepare as directed under Capsules, with Loperamide Hydrochloride.

Identification (1) Transfer a portion of the contents of Loperamide Hydrochloride Capsules, equivalent to about 10 mg of Loperamide Hydrochloride, to a stoppered vial, add 10 mL of methanol, shake for 5 minutes and filter. Use this solution as the test solution. Separately dissolve a portion of Loperamide Hydrochloride RS in methanol to render the concentration of 10 mg per mL and use this solution as the standard solution. Spot 10 μL of the test solution and 1 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate using a mixture of chloroform, methanol and formic acid (85 : 10 : 5) to a distance of about 15 cm, dry the plate between 40 °C and 60 °C and expose the plate to fumes of iodine: the R_f value of the spot obtained from the test solution corresponds to that obtained from the standard solution.

(2) The retention time of the major peak in the chromatogram of the test solution corresponds to that of the standard solution, as obtained in the Assay.

Dissolution Test Perform the test with 1 capsule of Loperamide Hydrochloride Capsules at 100 revolutions per minute according to Method 1 under the Dissolution Test. Mix 200 mL of 1 mol/L acetic acid TS and 600 mL of water, adjust pH to 4.70 ± 0.05 with 1 mol/L sodium hydroxide TS and dilute with water to make 1000 mL. Use 500 mL of this solution as a dissolution solution. Filter the dissolved solution after 30 minutes from the start of the test. Use the filtrate as the test solution. Separately, weigh Loperamide Hydrochloride RS, previously dried at 80 °C for 4 hours in vacuum and dissolve at the same concentration as the test solution and use this solution as the standard solution. Assay 50 μL each of the test solution and the standard solution as directed in the Assay under Loperamide Hydrochloride.
The dissolution rate of Loperamide Hydrochloride Capsules in 30 minutes is not less than 80 %.

Uniformity of Dosage Units It meets the requirement when the content uniformity test is performed as directed under the Assay.

Assay Transfer, as completely as possible, the contents of not less than 20 Loperamide Hydrochloride Capsules and determine the average weight per capsule. Transfer a portion of the powder, accurately weighed, equivalent to about 10 mg of Loperamide Hydrochloride in diluted methanol (7 in 10) to make exactly 50 mL and treat under the supersonic wave for 15 minutes and filter. Discard first 10 mL of the filtered solution and pipet 10.0 mL of the filtrate, add 4.0 mL of the internal standard solution and diluted methanol (7 in 10) to make exactly 50 mL and use this solution as the test solution. Separately, take accurately about 10 mg Loperamide Hydrochloride RS, previously dried for 4 hours at 80 °C in vacuum and dissolve in diluted methanol (7 in 10) to make exactly 50 mL. Pipet 10.0 mL of this solution, add 4.0 mL of the internal standard solution and diluted methanol (7 in 10) to make exactly 50

mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography. Calculate the ratios, Q_T and Q_S, of the peak areas of loperamide to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of loperamide hydrochloride } (C_{29}H_{33}ClN_2O_2\cdot HCl) = \text{Amount (mg) of Loperamide Hydrochloride RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—Weigh about 15 mg of propylparaben and dissolve in diluted methanol (7 in 10) in a volume of 50 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column, about 4 mm in internal diameter and 15 cm to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Mobile phase: Dissolve about 1.8 g of anhydrous monosodium phosphate in 300 mL of water, add 700 mL of methanol and titrate with phosphoric acid (1 in 100) to pH 7.0.

Flow rate: Adjust the flow rate so that the retention time of loperamide is about 9 minutes.

System suitability

System performance: When the procedure is run with 10 μL each of the standard solution according to the above conditions, internal standard and loperamide are eluted in this order with the resolution between their peaks being not less than 3.0.

Containers and Storage ***Containers***—Tight containers.

Loracarbef Capsules

Loracarbef Capsules contain not less than 90.0 % and not more than 110.0 % of the labeled amount of loracarbef ($C_{16}H_{16}ClN_3O_4$: 349.77).

Method of Preparation Prepare as directed under Capsules, with Loracarbef Hydrate.

Identification When proceed as directed in the Assay, the retention time of the principal peak from the test solution corresponds to that from the standard solution.

Purity ***Related substances*—**Weigh accurately the contents of not less than 5 Loracarbef Capsules. Weigh accurately a portion of the contents, equivalent to about 125 mg (potency) of loracarbef, add 20 mL of mobile phase A and sonicate to dissolve. To this solution, add the mobile phase to make exactly 25 mL, filter and use the filtrate as the test solution. Keep the test solution in a refrigerator and use within 24 hours. Separately, weigh accurately 0.1 mg (potency) of Loracarbef RS, add 10 mL of mobile phase A and use this solution as the standard solution. Perform the test with 20 μL each of mobile phase A, the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine each peak area in each solution by the automatic integration method and calculate the amount of each related substance: the amount of each related substance is not more than 1.0 % and the total amount of related substances is not more than 3.0 %. Of the peaks from the test solution, disregard any peaks that appear in mobile phase A.

$$\text{Content (\%) of each related substance} = 100 \times \frac{C_S}{C_T} \times \frac{A_i}{A_S}$$

C_S: Concentration [mg (potency)/mL] of loracarbef in the standard solution

C_T: Concentration [mg (potency)/mL] of loracarbef in the test solution

A_i: Peak area of each related substance in the test solution

A_S: Peak area of loracarbef in the standard solution

Operating conditions

Proceed as directed in the operating conditions in Purity (2) under Loracarbef Hydrate.

System suitability

Proceed as directed in the system suitability in Purity (2) under Loracarbef Hydrate.

Water Not more than 8.5 % (0.1 g, volumetric titration, direct titration).

Dissolution Test Perform the test with 1 capsule of Loracarbef Capsules at 50 revolutions per minute according to Method 2 under Dissolution Test, using 900 mL of water as the dissolution solution. Take the dissolved solution after 30 minutes from the start of the test and filter. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make V' mL so that each mL contains about 22 μg of (potency) of loracarbef according to the labeled amount and use this solution as the test solution. Separately, weigh accurately about 0.11 g (potency) of Loracarbef RS and add water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution at 260 nm as directed under Ultraviolet-visible Spectrophotometry and calculate the dissolution rate. The dissolution rate of Loracarbef Capsules in 30 minutes is not less than 75 %.

Dissolution rate (%) with respect to the labeled amount of loracarbef ($C_{16}H_{16}ClN_3O_4$)
= Amount [mg (potency)] of Loracarbef RS

$$\times \frac{A_T}{A_S} \times \frac{V}{V'} \times \frac{1}{C} \times 90$$

C: Labeled amount [mg (potency)] of loracarbef ($C_{16}H_{16}ClN_3O_4$) in 1 capsule

Uniformity of Dosage Units It meets the requirement when the test is performed according to the content uniformity test.

Assay Proceed as directed in the Assay under Loracarbef Hydrate. Weigh accurately the contents of not less than 20 Loracarbef Capsules. Weigh accurately a portion of the contents, equivalent to about 0.25 g (potency) according to the labeled amount, and add the internal standard solution to make 250 mL. Pipet 20 mL of this solution, make 100 mL and use this solution as the test solution. Separately, weigh accurately about 10 mg (potency) of Loracarbef RS, dissolve in the internal standard solution to make exactly 50 mL and use this solution as the standard solution.

Containers and Storage ***Containers*—**Tight containers.

Loracarbef for Syrup

Loracarbef for Syrup is a preparation for syrup, which is suspended before use. Loracarbef for Syrup contains not less than 90.0 % and not more than 115.0 % of the labeled amount of loracarbef ($C_{16}H_{16}ClN_3O_4$: 349.77).

Method of Preparation Prepare as directed under Syrups, with Loracarbef Hydrate.

Identification When proceed as directed in the Assay, the retention time of the principal peak from the test solution corresponds to that from the standard solution.

pH The pH of a solution obtained by suspending Loracarbef for Syrup according to the label is between 3.5 and 6.0.

Purity ***Related substances*—**Take exactly an amount of Loracarbef for Syrup, equivalent to 100 mg (potency) of loracarbef according to the labeled amount. Add mobile phase A, sonicate, add mobile phase A to make 25 mL, filter and use this solution as the test solution. Keep the test solution in a refrigerator and use within 24 hours. Separately, weigh accurately 0.1 mg (potency) of Loracarbef RS, add 10 mL of mobile phase A and use this solution as the standard solution. Perform the test with 20 μL each of mobile phase A, the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine each peak area in each solution by the automatic integration method and calculate the amount of each related substance: the amount of each related substance is not more than 1.0 % and the total amount of related substances is not more than 4.0 %. Of the peaks from the test solution, disregard any peaks that appear in mobile phase A.

Content (%) of each related substance

$$= 100 \times \frac{C_S}{C_T} \times \frac{A_i}{A_S}$$

C_S: Concentration [mg (potency)/mL] of loracarbef in the standard solution

C_T: Concentration [mg (potency)/mL] of loracarbef in the test solution

A_i: Peak area of each related substance in the test solution

A_S: Peak area of loracarbef in the standard solution

Operating conditions

Proceed as directed in the operating conditions in the Purity (2) under Loracarbef Hydrate.

System suitability

Proceed as directed in the system suitability in the Purity (2) under Loracarbef Hydrate.

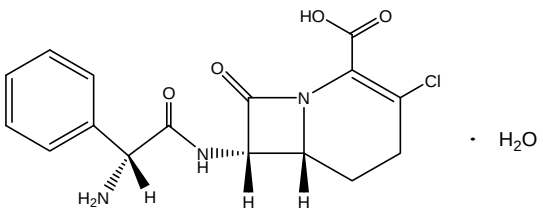
Water Not more than 2.0 % (0.5 g, volumetric titration, direct titration).

Uniformity of Dosage Units (divided) It meets the requirement.

Assay Proceed as directed in the Assay under Loracarbef Hydrate. Weigh accurately an amount of Loracarbef for Syrup, equivalent to about 0.2 g (potency) according to the labeled potency, and add the mobile phase to make exactly 100 mL. To exactly 10 mL of this solution, add the mobile phase to make exactly 100 mL, filter and use the filtrate as the test solution. Separately, weigh accurately about 10 mg (potency) of Loracarbef RS, dissolve in the mobile phase to make exactly 50 mL and use this solution as the standard solution.

Containers and Storage ***Containers*—**Tight containers.

Loracarbef Hydrate



Loracarbef $C_{16}H_{16}ClN_3O_4 \cdot H_2O$: 367.79

(6*R*,7*S*)-7-[[(2*R*)-2-Amino-2-phenylacetyl]amino]-3-chloro-8-oxo-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid hydrate [*121961-22-6*]

Loracarbef Hydrate contains not less than 960 μg (potency) and not more than 1020 μg (potency) per mg of loracarbef ($C_{16}H_{16}ClN_3O_4$: 349.77), calculated on the anhydrous basis.

Description Loracarbef Hydrate appears as white or pale gray crystalline powder.
Loracarbef Hydrate is slightly soluble in water and very slightly soluble in methanol, in octanol, in 2-propanol, in acetonitrile, in acetone, in chloroform, in ethyl acetate, in ether, in cyclohexane and in toluene.
Loracarbef Hydrate dissolves slightly in phosphate buffer (pH 7.0).
Loracarbef Hydrate dissolves in hydrochloric acid buffer (pH 1.2).

Identification (1) Determine the infrared spectra of Loracarbef Hydrate and Loracarbef Hydrate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) When proceed as directed in the Assay, the retention time of the principal peak from the test solution corresponds to that from the standard solution.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{20}$: + 27 ~ + 33° (0.1 g calculated on the anhydrous basis, 0.1 mol/L hydrochloric acid TS, 10 mL, 100 mm)

pH The pH of a solution obtained by suspending 1.0 g of Loracarbef Hydrate in 10 mL of water is between 3.5 and 5.5.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Loracarbef Hydrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (20 ppm).

(2) ***Related substances***—Weigh accurately about 50 mg of Loracarbef Hydrate, add mobile phase A to make exactly 10 mL and use this solution as the test solution. Separately, weigh accurately a suitable amount of Loracarbef RS, dissolve in mobile phase A to make a solution containing 0.01 mg per mL and use this solution as the standard solution. Perform the test with 20 μL each of mobile phase A, phenylglycine solution, the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine each peak area by the automatic integration method and calculate the amount of related substances. Of the peaks from the test solution, disregard those that are equal to the peak of mobile phase A and calculate the amount of those that are equal to the peak of the phenylglycine solution. Calculate the amount of phenylglycine according to the following equation: not more than 0.15 %. The amount of any other related substance is not more than 0.5 % and the total amount of related substances is not more than 2.0 %.

$$\text{Content (\%) of each related substance} = \frac{C_S}{C_T} \times \frac{A_i}{A_S}$$

C_S: Concentration (mg/mL) of loracarbef in the standard solution

C_T: Concentration (mg/mL) of loracarbef in the test solution

A_i: Peak area of each related substance in the test solution

A_S: Peak area of loracarbef in the standard solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: Control the step or concentration gradient by mixing mobile phases A and B as directed in the following table.

Mobile phase A: Dissolve 6.9 g of ammonium dihydrogen phosphate in 1960 mL of water, adjust the pH to 2.5 with phosphoric acid, add 40 mL of acetonitrile and mix.

Mobile phase B: Dissolve 6.9 g of ammonium dihydrogen phosphate in 600 mL of water, adjust the pH to 2.5 with phosphoric acid, add 1400 mL of acetonitrile and mix.

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-4	100	0
4-13.5	100→85.5	0→14.5
13.5-21.0	85.5→0	14.5→100
21.0-22.5	0	100

Flow rate: 2 mL/minute

System suitability

System performance: When the procedure is run with 20 μL of the system suitability solution under the above operating conditions, the relative retention time of cefaclor and loracarbef is 0.9 and 1.0, respectively, with the resolution between their peaks being between 4.0 and 8.0 and the symmetry factor of loracarbef being not more than 1.3. The recovery rate of loracarbef from this solution is between 95 % and 105 %.

Recovery rate (%) of loracarbef

$$= \frac{C_S}{C_L} \times \frac{A_L}{A_S} \times 100$$

C_S: Concentration (mg/mL) of loracarbef in the standard solution

C_L: Concentration (mg/mL) of loracarbef in the system suitability solution

A_L: Peak area of loracarbef in the system suitability solution

A_S: Peak area of loracarbef in the standard solution

Phenylglycine solution—Weigh accurately a suitable amount of phenylglycine and dissolve in mobile phase A to make a solution containing 0.0075 mg per mL.

System suitability solution—Dissolve Loracarbef RS and Cefaclor RS in mobile phase A to make a solution containing 0.01 mg/mL of each and use this solution as the system suitability solution.

Water 3.5 % ~ 6.0 % (0.2 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 10 mg (potency) each of Loracarbef Hydrate and Loracarbef RS, dissolve each in the mobile phase to make exactly 50 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the ratios, Q_T and Q_S, of the peak area of loracarbef to that of the internal standard in the test solution and the standard solution.

$$\text{Amount [μg (potency)] of loracarbef } (C_{16}H_{16}ClN_3O_4) = \text{Amount [μg (potency)] of Loracarbef RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—Dissolve 0.2 g of 1-naphthalenesulfonate and 13.2 g of ammonium monohydrogen phosphate in water to make 1000 mL and adjust the pH to 6.5 with phosphoric acid.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength 265 nm)

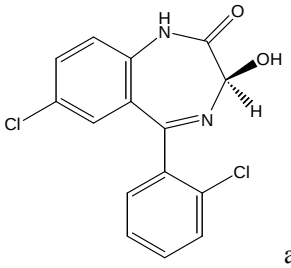
Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Dissolve 1.0 g of 1-pentanesulfonic acid sodium in 780 mL of water, add 10 mL of triethylamine, adjust the pH to 2.5 with phosphoric acid and add 220 mL of methanol.

Flow rate: 1.5 mL/minute

Containers and Storage ***Containers***—Tight containers.

Lorazepam



and enantiomer

$C_{15}H_{10}Cl_2N_2O_2$: 321.16

(*RS*)-7-Chloro-5-(2-chlorophenyl)-3-hydroxy-1,3-dihydro-1,4-benzodiazepin-2-one [*846-49-1*]

Lorazepam, when dried, contains not less than 98.5 % and not more than 101.0 % of lorazepam ($C_{15}H_{10}Cl_2N_2O_2$).

Description Lorazepam is a white, crystalline powder and is odorless.

Lorazepam is sparingly soluble in ethanol (95) or in acetone, slightly soluble in ether and practically insoluble in water.

Lorazepam is gradually affected by light.

Identification (1) Take 20 mg of Lorazepam, add 15 mL of dilute hydrochloric acid, boil for 5 minutes and cool: the solution responds to the Qualitative Tests for primary aromatic amines.

(2) Determine the absorption spectra of solutions of Lorazepam and Lorazepam RS in ethanol (95) (1 in 200000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Lorazepam and Lorazepam RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(4) Perform the test with Lorazepam as directed under Flame Coloration Test (2): a green color is observed.

Absorbance $E_{1\,cm}^{1\%}$ (229 nm): 1080 ~ 1126 (after drying, 1 mg, ethanol (95), 200 mL).

Purity (1) ***Chloride***—Take 1.0 g of Lorazepam, add 50 mL of water, allow to stand for 1 hour with occasional shaking and filter. To 25 mL of the filtrate, add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than

0.014 %).

(2) ***Heavy metals***—Proceed with 1.0 g of Lorazepam according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Lorazepam according to Method 3 and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 0.10 g of Lorazepam in 20 mL of ethanol (95) and use this solution as the test solution. Pipet 1.0 mL of the test solution, add ethanol (95) to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, dioxane and glacial acetic acid (91 : 51 : 4) to a distance of about 15 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1g, in vacuum, 105 °C, 3 hours).

Residue on Ignition Not more than 0.3 % (1 g).

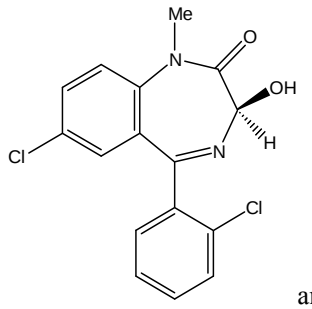
Assay Weigh accurately about 0.4 g of Lorazepam, previously dried, dissolve in 50 mL of acetone and titrate with 0.1 mol/L tetrabutylammonium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L tetrabutylammonium hydroxide VS = 32.116 mg of $C_{15}H_{10}Cl_2N_2O_2$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Lormetazepam



and enantiomer

$C_{16}H_{12}Cl_2N_2O_2$: 335.19

7-Chloro-5-(2-chlorophenyl)-3-hydroxy-1-methyl-1*H*-benzo[1,4]diazepin-2(3*H*)-one [*848-75-9*]

Lormetazepam contains not less than 99.0 % and not more than 101.0 % of lometrazepam ($C_{16}H_{12}Cl_2N_2O_2$), calculated on the dried basis.

Description Lormetazepam appears as white crystalline powder.
Lormetazepam is soluble in methanol or in ethanol and practically insoluble in water.

Identification (1) Determine the infrared spectra of Lormetazepam and Lormetazepam RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) When proceed as directed in the Related substances under the Purity, the retention time of the Lormetazepam peak from the test solution corresponds to that from the standard solution (2).

Purity ***Related substances***—Weigh accurately 0.250 g of Lormetazepam, add 70 % methanol to make exactly 100 mL and use this solution as the test solution (1). Pipet accurately 1 mL of this solution, add 70 % methanol to make exactly 100 mL, pipet 10 mL of this solution, add 70 % methanol to make exactly 50 mL and use this solution as the test solution (2). Pipet accurately 25 mL of the test solution (2), add 70 % methanol to make exactly 50 mL and use this solution as the test solution (3). Separately, weigh accurately 5 mg of Lormetazepam RS and add 70 % methanol to make exactly 100 mL. Pipet 5 mL of this solution, add 70 % methanol to make 50 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solutions (1), (2) and (3), and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the area of the peaks from these solutions by the automatic integration method: the area of each peak other than the principal peak obtained from the test solution (1) is not more than the area of the principal peak obtained from the test solution (2) (0.2 %), the number of peaks having the area greater than the area of the principal peak from the test solution (3) is not more than 2 from the test solution (1) and total area of these peaks from the test solution (1) is not more than 2.5 times the area of the principal peak from the test solution (2) (0.5 %).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of phosphate buffer and

methanol (52:48).

Flow rate: 2 mL/minute.

System suitability

System performance: Weigh 5 mg of Lormetazepam RS, add 70 % methanol to make 100 mL, pipet 5 mL of this solution and add 70 % methanol to make 50 mL. Mix 25 mL of this solution and 25 mL of a solution containing 5 μg Lorazepam per mL, prepared by dissolving Lorazepam RS in 70 % methanol. When the procedure is run with 20 μL of this solution under the above operating conditions, the resolution between two principal peaks is not less than 4.

Phosphate buffer—Dissolve 4.91 g of sodium dihydrogen phosphate and 0.633 g of disodium hydrogen phosphate in water to make 1000 mL.

Loss on Drying Not more than 1.0 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

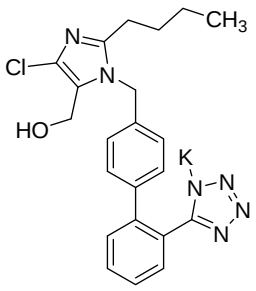
Assay Weigh accurately about 0.5 g of Lormetazepam, dissolve in 50 mL of nitroethane and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 33.52 mg of $C_{16}H_{12}Cl_2N_2O_2$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Losartan Potassium



$C_{22}H_{22}ClKN_6O$: 461.00

Potassium [2-butyl-5-chloro-3-[[4-[2-(1,2,3-triaza-4-azanidacyclopenta-2,5-dien-5-yl)phenyl]phenyl]methyl]imidazol-4-yl]methanol [*124750-99-8*]

Losartan Potassium contains not less than 98.5 % and not more than 101.0 % of losartan potassium ($C_{22}H_{22}ClKN_6O$), calculated on the anhydrous basis.

Description Losartan Potassium appears as white crystalline powder.

Losartan Potassium is very soluble in water and freely soluble in methanol or in ethanol (99.5).

Identification (1) Determine the absorption spectra of the solutions of Losartan Potassium and Losartan Potassium RS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Losartan Potassium and Losartan Potassium RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Losartan Potassium responds to the Qualitative Tests (1) for potassium salt.

(4) Perform the test with Losartan Potassium as directed under Flame Coloration Test (2): a green color is observed.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Losartan Potassium according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Dissolve 30 mg of Losartan Potassium in 100 mL of methanol and use this solution as the test solution. Pipet 1 mL of this solution, add methanol to make exactly 100 mL and use this solution as the standard solution. Perform the test with exactly 10 μL of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peaks of the solvent and losartan obtained from the test solution is not larger than 1/10 times the peak area of losartan from the standard solution, and the total area of the peaks other than the peak of losartan from the test solution is not larger than 3/10 times the peak area of losartan from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column 4.0 mm in internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Control the step or concentration gradient by mixing mobile phases A and B as directed in the following table.

Mobile phase A: Diluted phosphoric acid (1 in 1000)

Mobile phase B: Acetonitrile

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-25	75→10	25→90
25-35	10	90

Flow rate: 1.0 mL/minute

System suitability

Test for required detectability: Pipet 1 mL of the standard solution and add methanol to make exactly 10 mL. Confirm that the peak area of losartan obtained from 10 μL of this solution is equivalent to 7 % to 13 % of that of losartan from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of losartan is not less than 10000 with the symmetry factor being not more than 1.3.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of losartan is not more than 2.0 %.

Time span of measurement: 35 minutes after injection of the test solution

Water Not more than 0.5 % (0.25 g, volumetric titration, direct titration).

Assay Weigh accurately about 25 mg each of Losartan Potassium and Losartan Potassium RS (determine the water content), dissolve each in methanol to make exactly 100 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine the peak areas, A_T and A_S, of losartan in each solution.

Amount (mg) of losartan potassium ($C_{22}H_{22}ClKN_6O$) = Amount (mg) of Losartan Potassium RS, calculated on the anhydrous basis $\times \frac{A_T}{A_S}$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column 4.0 mm in internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35 °C

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3:2)

Flow rate: Adjust the flow rate so that the retention time of losartan is about 6 minutes.

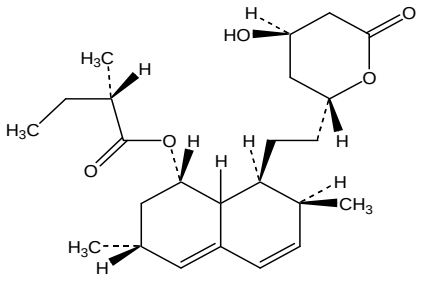
System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of losartan is not less than 5500 with the symmetry factor being not more than 1.4.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation ofo the peak area of losartan is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Lovastatin



$C_{24}H_{36}O_5$: 404.54

[(1*S*,3*R*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-Hydroxy-6-oxooxan-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl](2*S*)-2-methyl butanoate [*75330-75-5*]

Lovastatin contains not less than 98.5 % and not more than 101.0 % of lovastatin ($C_{24}H_{36}O_5$), calculated on the dried basis.

Description Lovastatin is a white, crystalline powder. Lovastatin is soluble in acetone, sparingly soluble in ethanol (95) and practically insoluble in water.

Identification (1) Dissolve 10 mg each of Lovastatin and Lovastatin RS in acetone to make 100 mL. Pipet 5 mL of the solutions, add acetonitrile to make exactly 100 mL, and use these solutions as the test solution and the standard solution. Determine the absorption spectra of the solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Lovastatin and Lovastatin RS as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +324 ~ +338° (0.125 g previously dried, acetonitrile, 25 mL, 100 mm).

Purity (1) ***Heavy metals***—Proceed the test with 1.0 g of Lovastatin according to Method 2. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Lovastatin related substance I***—Weigh accurately about 25 mg, dissolve in acetonitrile to make exactly 25 mL, and use this solution as the test solution. Separately, weigh accurately about 10.0 mg of Lovastatin RS, and dissolve in acetonitrile to make 100 mL. Pipet 2.0 mL of this solution, dilute with acetonitrile to 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Based on the peak areas of Lovastatin and lovastatin related substance I (dihydrolovastatin), calculate the percentage of the related compound I in Lovastatin according to the following the formula (not more than 1.0 %):

$$\text{Amount (\%) of lovastatin related substance I} = 2.5 \times F \times \frac{C}{W} \times \frac{A_T}{A_S}$$

F: Response factor for the related compound A (1.6);

C: Concentration of Lovastatin RS (μg/mL) in the standard solution

W: Amount (mg) of Lovastatin in the test solution

A_T: Peak area of lovastatin related compound I from the test solution

A_S: Peak area of lovastatin obtained from the standard solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 200 nm)

Column: A stainless steel column 4.6 mm in internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Flow rate: 1.5 mL/min.

Mobile phase: a mixture of acetonitrile and 0.01 mol/L phosphoric acid (13:7).

System suitability

System performance: Dissolve 10 mg each of Lovastatin RS and lovastatin related substance RS in acetonitrile to make 100 mL. Pipet 2.0 mL of this solution, and add acetonitrile to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, the relative retention times are about 1.0 for lovastatin and 1.3 for lovastatin related compound I with the resolution between these peaks being not less than 6.0.

System repeatability: When the test is run 6 times with 10 μL each of the standard solution, the relative standard deviation of the peak area of lovastatin is not more than 5.0 %.

(3) ***Other related substances***—Weigh accurately about 25 mg of Lovastatin, dissolve in acetonitrile to make exactly 25 mL, and use this solution as the test solution. Separately, weigh accurately about 10 mg of Lovastatin RS and dissolve in acetonitrile to make exactly 100 mL. Pipet 2 mL of this solution, add acetonitrile to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, measure all the peak areas by automatic integration, and calculate the percentages of related substances by the below formula. The percentage of each related substance is not more than 0.2 %, and the total percentage of all related substances is not more than 1.0 %. For this calculation, it is allowed to exclude any related substances with less than 0.04 %.

$$\text{Amount (\%) of related substances} = 2.5 \times F \times \frac{C}{W} \times \frac{A_T}{A_S}$$

F: Response factor for each related substance (1.4 for a related substance with the relative retention time of 0.73; 1.0 for any other related substances).

C: Concentration of Lovastatin RS (μg/mL) in the standard solution.

W: Amount (mg) of Lovastatin in the test solution.

A_T: Peak area of each related substance from the test solution.

A_S: Peak area of lovastatin obtained from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 238 nm)

Column: A stainless steel column 4.0 mm in internal diameter and 12.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Flow rate: 1.5 mL/min

Mobile phase: Use variable mixtures of solution A and solution B, and program the chromatograph as follows.

Mobile phase A: Adjust the pH of 0.001 mol/L phosphoric acid solution to 4.0 with 1 mol/L sodium hydroxide.

Mobile phase B: Acetonitrile.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0–2	60	40

2–5	60→45	40→55
5–8	45	55
8–16	45→10	55→90
16–25	10	90
25–27	10→60	90→40
27–35	60	40

System suitability

System performance: Dissolve 10 mg each of Lovastatin RS and compactin in acetonitrile to make 100 mL. Pipet 2.0 mL of this solution, and add acetonitrile to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the relative retention times are about 1.0 for lovastatin and 0.85 for compactin with the resolution between these peaks being not less than 3.5.

System repeatability: When the test is run 6 times with 10 μL each of the standard solution, the relative standard deviation of the peak area of lovastatin is not more than 5.0 %.

Loss on Drying Not more than 0.3 % (1 g, vacuum, 60 °C, 6 hours).

Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 30 mg each of Lovastatin and Lovastatin RS, dissolve in acetonitrile to make exactly 100 mL, and use these solutions as the test solution and the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, A_T and A_S, of the peak area of lovastatin from the test solution to that from the standard solution.

$$\text{Amount (mg) of lovastatin } (C_{24}H_{36}O_5) = \text{Amount (mg) of Lovastatin RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 238 nm).

Column: A stainless steel column 4.6 mm in internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Flow rate: 1.5 mL/min.

Mobile phase: a mixture of acetonitrile and 0.1 % phosphoric acid (65:35).

System suitability

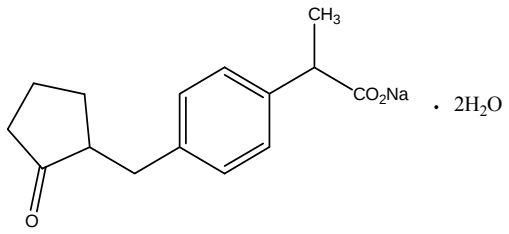
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the column efficiency is not less than 3000 theoretical plates, and the symmetry factor is not more than 1.4.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Under nitrogen atemosphere, and in a cold place.

Loxoprofen Sodium Hydrate



$C_{15}H_{17}NaO_3.2H_2O$: 304.31

sodium(*RS*)-2-[4-[(2-oxocyclopentyl)methyl]phenyl] propanoate dihydrate [*80382-23-6*]

Loxoprofen Sodium Hydrate contains not less than 98.5 % and not more than 101.0 % of loxoprofen sodium ($C_{15}H_{17}NaO_3$: 268.28), calculated on the anhydrous basis.

Description Loxoprofen Sodium Hydrate appears as white to yellowish-white crystals or crystalline powder. Loxoprofen Sodium Hydrate is very soluble in water or in methanol, soluble in ethanol (95) and practically insoluble in ether.

A solution of Loxoprofen Sodium Hydrate (1 in 20) shows no optical rotation.

pH—The pH of a solution of Loxoprofen Sodium Hydrate in freshly boiled and cooled water (1 in 20) is between 6.5 and 8.5.

Identification (1) Determine the absorption spectra of solutions of Loxoprofen Sodium Hydrate and Loxoprofen Sodium Hydrate RS (1 in 55000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Loxoprofen Sodium Hydrate and Loxoprofen Sodium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Loxoprofen Sodium Hydrate (1 in 10) responds to the Qualitative Tests for sodium salt.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Loxoprofen Sodium Hydrate in 10 mL of water: the solution is clear and colorless to pale yellow. The color is not darker than that of diluted Color Matching Fluid A (1 in 2).

(2) ***Heavy metals***—Proceed with 2.0 g of

Loxoprofen Sodium Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Dissolve 1.0 g of Loxoprofen Sodium Hydrate in 10 mL of methanol, and use this solution as the test solution. Pipet 1 mL of the test solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standrd solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1,2-dichloroethane and glacial acetic acid (9:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Water Not less than 11.0 % and not more than 13.0 % (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately about 60 mg of Loxoprofen Sodium Hydrate, and dissolve in diluted methanol (3 in 5) to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution, add diluted methanol (3 in 5) to make 100 mL, and use this solution as the test solution. Separately, weigh accurately about 50 mg of Loxoprofen RS, previously dried in a desiccator (in vacuum, 60 °C) for 3 hours, and dissolve in diluted methanol (3 in 5) to make exactly 100 mL. Pipet 5 mL of this solution, proceed in the same manner as directed for the preparation of the test solution, and use so obtained solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of loxoprofen to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of loxoprofen sodium } (C_{15}H_{17}NaO_3) = \text{Amount (mg) of Loxoprofen RS} \times \frac{Q_T}{Q_S} \times 1.0892$$

Internal Standard Solution—A solution of ethyl benzoate in diluted methanol (3 in 5) (1 in 50000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 222 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: A mixture of methanol, water, acetic acid (100) and triethylamine (600:400:1:1).

Flow rate: Adjust the flow rate so that the retention time of loxoprofen is about 7 minutes.

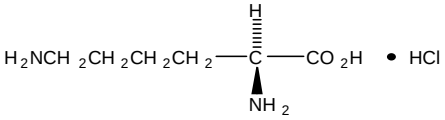
System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, loxoprofen and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of loxoprofen to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

L-Lysine Hydrochloride



Lysine Hydrochloride $C_6H_{14}N_2O_2 \cdot HCl$: 182.65

(2*S*)-2,6-Diaminohexanoic acid hydrochloride
[*657-27-2*]

L-Lysine Hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of L-lysine hydrochloride ($C_6H_{14}N_2O_2 \cdot HCl$).

Description L-Lysine Hydrochloride appears as white powder, is odorless and has a slight, characteristic taste.
L-Lysine Hydrochloride is freely soluble in water or in formic acid, very slightly soluble in ethanol (95) and practically insoluble in ether.

Specific Optical Rotation $[\alpha]_D^{20}$: +19.0 ~ +21.5° (2 g after drying, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

Identification (1) Determine the infrared spectra of L-Lysine Hydrochloride and L-Lysine Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears between the spectra, dissolve L-Lysine Hydrochloride in water, evaporate the water to dryness at 60 °C, and repeat the test with the residue.

(2) A solution of L-Lysine Hydrochloride (1 in 10) responds to the Qualitative Tests for chloride.

pH Dissolve 1.0 g of L-Lysine Hydrochloride in 10

mL of water: the pH of this solution is between 5.0 and 6.0.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of L-Lysine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) ***Sulfate***—Perform the test with 0.6 g of L-Lysine Hydrochloride. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028 %).

(3) ***Ammonium***—Perform the test with 0.25 g of L-Lysine Hydrochloride. Prepare the control solution with 5.0 mL of standard ammonium solution (not more than 0.02 %).

(4) ***Heavy metals***—Proceed with 2.0 g of L-Lysine Hydrochloride according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(5) ***Iron***—Dissolve 0.333 g of L-Lysine Hydrochloride in water to make 45 mL, add 2 mL of hydrochloric acid and use this solution as the test solution. To 1.0 mL of iron standard solution, add water to make 45 mL, add 2 mL of hydrochloric acid and use this solution as the standard solution. To each of the test solution and the standard solution, add 50 mg of ammonium peroxydisulfate and 3 mL of 30 % ammonium thiocyanate solution and mix: the color obtained from the test solution is not more intense than that from the standard solution (not more than 30 ppm).

(6) ***Arsenic***—Prepare the test solution with 1.0 g of L-Lysine Hydrochloride according to Method 1 and perform the test (not more than 2 ppm).

(7) ***Related substances***—Dissolve 0.10 g of L-Lysine Hydrochloride in 25 mL of water and use this solution as the test solution. Pipet 1 mL of the test solution, add water to make exactly 50 mL, pipet 5 mL of this solution, add water to make exactly 20 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol and ammonia solution (28) (67 : 33) to a distance of about 10 cm and dry the plate at 100 °C for 30 minutes. Spray evenly the plate with a solution of ninhydrin in acetone (1 in 50) and heat at 80 °C for 5 minutes: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 1.0 % (1 g, 105 °C, 3 hours).

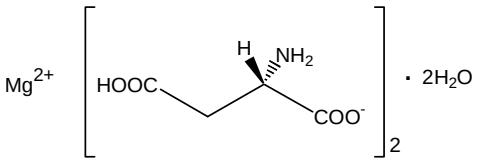
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately 0.1 g of L-Lysine Hydrochloride, previously dried, dissolve in 2 mL of formic acid, add exactly 25 mL of 0.1 mol/L perchloric acid VS and heat in a water-bath for 30 minutes. After cooling, add 45 mL of acetic acid (100) and titrate the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 9.132 mg of $C_6H_{14}N_2O_2 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Magnesium Aspartate Hydrate



$C_8H_{12}O_8N_2Mg \cdot 2H_2O$: 324.53

Magnesium (2*S*)-2-amino-4-hydroxy-4-oxo-butanoate dihydrate

Magnesium Aspartate Hydrate contains not less than 98.0 % and not more than 102.0 % of magnesium aspartate ($C_8H_{12}O_8N_2Mg$: 288.49), calculated on the anhydrous basis.

Description Magnesium Aspartate Hydrate appears as white crystalline powder or colorless crystals.
Magnesium Aspartate Hydrate is freely soluble in water.

Identification (1) Ignite about 15 mg of Magnesium Aspartate Hydrate until a white residue is obtained, dissolve the residue in 1 mL of dilute hydrochloric acid, neutralize to red litmus paper by the addition of dilute sodium hydroxide TS and filter if necessary. Upon the addition of 6 mol/L ammonia water to this solution, white precipitate is formed and the precipitate is dissolved with the addition of 10.7 w/v % ammonium chloride solution, and the addition of 9 w/v % disodium hydrogen phosphate solution form a white crystalline precipitate.

(2) Dissolve 0.10 g of the Magnesium Aspartate Hydrate in water to make 10 mL, dilute 1 mL of this solution with water to make 50 mL, and use this solution as the test solution. Separately, dissolve 10 mg of Magnesium Aspartate Hydrate RS in water to make 50 mL, and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with the upper layer of the mixture of 1-

butanol, water and acetic acid (100) (60 : 20 : 20) to a distance of about 15 cm and air-dry the plate. Spray evenly ninhydrin TS on the plate and heat at 105 °C for 15 minutes: the color and R_f value of both the principal spots from the test solution and the standard solution are the same.

Specific Optical Rotation $[\alpha]_D^{20}$: +20.5 ~ +23.0º (0.50 g, a solution prepared by mixing 51.5 g of hydrochloric acid and water to make 100 mL, 25 mL, 100 mm).

pH The pH of a solution of Magnesium Aspartate Hydrate in water (25 in 100) is between 6.0 and 8.0.

Purity (1) ***Clarity and color of solution***—A 100 mL solution of 2.5 g Magnesium Aspartate Hydrate in water is colorless and clear.

(2) ***Chlorides***—Perform the test with 1.0 g of Magnesium Aspartate Hydrate. Prepare the control solution with 0.564 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.02 %).

(3) ***Sulfates***—Perform the test with 0.5 g of Magnesium Aspartate Hydrate. Prepare the control solution with 0.52 mL of 0.005 mol/L sulfuric acid VS (not more than 0.05 %).

(4) ***Ammonium***—Perform the test with 0.25 g of Magnesium Aspartate Hydrate. Prepare the control solution with 5 mL of ammonium standard solution (not more than 0.02 %).

(5) ***Heavy metals***—Proceed with 2.0 g of Magnesium Aspartate Hydrate according to Method 1. and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(6) ***Iron***—In a separatory funnel, dissolve 0.20 g of Magnesium Aspartate Hydrate in 10 mL of dilute hydrochloric acid. Extract three quantities, each of 10 mL, of 4-methyl-2-pentanone, shaking for 3 minutes each time. To the combined organic layers add 10 mL of water and shake for 3 minutes. Put the aqueous layer in a Nessler tube, add 2 mL of 20 w/v % citric acid solution and 0.1 mL of mercaptoacetic acid, alkalinize with 10 mol/L ammonia water, then add water to 20 mL, and allow to stand for 5 minutes: the color appeared is not darker than the color of the standard solution obtained from the same process with 10 mL of the standard iron solution (1 in 10) (not more than 50 ppm).

(7) ***Ninhydrin-positive substances***—Dissolve 0.10 g of Magnesium Aspartate Hydrate in water to make 10 ml. Dilute 1 mL of this solution with water to make 50 mL and use this solution as the test solution (1). Dilute 1 mL of the test solution (1) with water to make 50 mL and use this solution as the test solution (2). Dilute 5 mL of the test solution (2) with water to make 20 mL and use this solution as the standard solution (1). Separately, dissolve 10 mg of Magnesium Aspartate Hydrate RS and 10 mg of Glutamic Acid RS in water to make 25 mL and use this solution as the standard solution (2). Perform the test with the test solutions and the standard solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution (1) and the standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with the upper layer of the mixture of 1-butanol, water and acetic acid (100) (60 : 20 : 20) to a distance of about 15 cm and air-dry the plate. Spray evenly ninhydrin TS on the plate and heat at 105 ºC for 15 minutes: Any spot from the test solution (1), other than the principal spot, is not more intense than the spot from the standard solution (1) (0.5 %). The test is not valid unless the chromatogram obtained with the standard solution (2) shows two clearly separated principal spots.

Water 10.0 ~ 14.0 % (0.1 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.26 g of Magnesium Aspartate Hydrate, dissolve in 10 mL of water, add 10 mL of ammonia·ammonium chloride buffer solution (pH 10.0), and titrate with 0.1 mol/L disodium ethylenediaminetetraacetate VS until the color of the solution changes from violet to blue (indicator: about 50 mg of eriochrome black T·sodium chloride indicator). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L
disodium ethylenediaminetetraacetate VS
= 28.853 mg of $C_8H_{12}MgN_2O_8$.

Containers and Storage ***Containers***—Well-closed containers.

Magnesium Carbonate

[*546-93-0*]

Magnesium Carbonate is a basic hydrated magnesium carbonate or a normal hydrated magnesium carbonate. Magnesium Carbonate contains not less than 40.0 % and not more than 44.0 % of magnesium oxide (MgO: 40.30).
Heavy Magnesium Carbonate may be used as a commonly used name for Magnesium Carbonate which shows the height of the precipitate below the 12.0 mL graduation line in the Precipitation Test.

Description Magnesium Carbonate is a white, friable masses or powder and is odorless.
Magnesium Carbonate is practically insoluble in water, in ethanol (95), in ether or in 1-propanol.
Magnesium Carbonate dissolves in dilute hydrochloric acid with effervescence.
Saturated solution of Magnesium Carbonate is alkaline.

Identification (1) Dissolve 1 g of Magnesium Carbonate in 10 mL of dilute hydrochloric acid, boil, then cool, neutralize with sodium hydroxide TS and filter, if

necessary: the solution responds to the Qualitative Tests for magnesium salt.

(2) Magnesium Carbonate responds to the Qualitative Tests (1) for carbonate.

Purity (1) ***Soluble salts***—To 2.0 g of Magnesium Carbonate, add 40 mL of 1-propanol and 40 mL of water, heat to boil with constant stirring, cool and filter. Wash the residue with water, combine the washings with the filtrate and add water to make exactly 100 mL. Evaporate 50 mL of the solution on a water-bath to dryness and dry at 105 °C for 1 hour: the residue is not more than 10.0 mg.

(2) ***Heavy metals***—Moisten 1.0 g of Magnesium Carbonate with 4 mL of water, dissolve by addition of 10 mL of dilute hydrochloric acid and evaporate on a water-bath to dryness. Dissolve the residue in 35 mL of water, 2 mL of dilute acetic acid, 1 drop of ammonia TS, filter, if necessary, wash the filter paper with water, combine the washings with the filtrate and add water to make 50 mL and perform the test. Prepare the control solution as follows: Evaporate 10 mL of dilute hydrochloric acid on a water-bath to dryness, add 2 mL of dilute acetic acid and 3.0 mL of standard lead solution and dilute with water to make 50 mL (not more than 30 ppm).

(3) ***Iron***—Prepare the test solution with 0.10 g of Magnesium Carbonate according to Method 1 and perform the test according to Method A. Prepare the control solution with 2.0 mL of standard iron solution (not more than 200 ppm).

(4) ***Arsenic***—Prepare the test solution with 0.40 g of Magnesium Carbonate, previously moistened with 1.5 mL of water, add 3.5 mL of dilute hydrochloric acid and perform the test (not more than 5 ppm).

(5) ***Calcium oxide***—Weigh accurately about 0.6 g of Magnesium Carbonate and dissolve in 35 mL of water and 6 mL of dilute hydrochloric acid. Add 250 mL of water and 5 mL of a solution of L-tartaric acid (1 in 5), then add 10 mL of a solution of 2,2',2''-nitryltri-ethanolamine (3 in 10) and 10 mL of 8 mol/L potassium hydroxide TS, allow to stand for 5 minutes and titrate with 0.01 mol/L disodium ethylenediaminetetraacetate VS until the color of the solution changes from red-purple to blue (indicator: 0.1 g of NN indicator). Perform a blank determination and make any necessary correction.
The content of calcium oxide (CaO: 56.08) is not more than 0.6 %.

Each mL of 0.01 mol/L
disodium ethylenediaminetetraacetate VS
= 0.5608 mg of CaO

(6) ***Acid-insoluble substances***—Mix 5.0 g of Magnesium Carbonate and 75 mL of water, add 10 mL of hydrochloric acid drop-wise while stirring, boil for 5 minutes and cool. Collect the insoluble residue using filter paper for Assay, wash well with water until the last washing shows no turbidity with silver nitrate TS and ignite the residue together with the filter paper: the residue is not more than 2.5 mg.

Precipitation Test Transfer 1.0 g of Magnesium Carbonate, previously sifted through a No. 100 sieve to a glass-stoppered measuring cylinder with a 50 mL graduation line at 150 mm from the bottom and add water to make 50 mL, Shake vigorously for exactly 1 minute, allow to stand for 15 minutes and measure the height of the precipitate (in graduation in mL).

Microbial Limit The total aerobic microbial count is not more than 1000 CFU/g, the total combined yeasts/mould count is not more than 100 CFU/g, and *Escherichia coli*, *Salmonell* species, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are not observed.

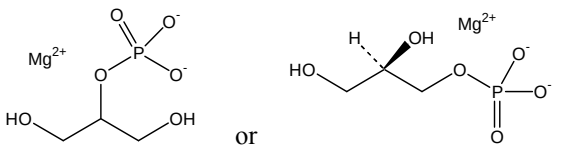
Assay Weigh accurately about 0.4 g of Magnesium Carbonate, dissolve in 10 mL of water and 3.5 mL of dilute hydrochloric acid and add water to make exactly 100 mL. Pipet 25.0 mL of the solution, add 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7 and titrate with 0.05 mo1/L disodium ethylenediaminetetraacetate VS (indicator: 40 mg of eriochrome black T-sodium chloride indicator). Perform a blank determination and make any necessary correction. From the volume of 0.05 mo1/L disodium ethylenediaminetetraacetate VS consumed deduct the volume of 0.05 mol/L disodium ethylenediaminetetraacetate VS corresponding to the content of Calcium Oxide (CaO) obtained in the Purity (5).

Each mL of 0.05 mol/L
disodium ethylenediaminetetraacetate VS
= 2.0152 mg of MgO

Each mg of calcium oxide (CaO) = 0.36 mL of
0.05 mol/L disodium ethylenediaminetetraacetate VS

Containers and Storage ***Containers***—Well-closed containers.

Magnesium Glycerophosphate



and enantiomer

$C_3H_7MgO_6P$: 194.36

Magnesium 2,3-dihydroxypropyl phosphate

Magnesium Glycerophosphate contains not less than 11.0 % and not more than 12.5 % of Mg, calculated on

the dried basis.

Description Magnesium Glycerophosphate appears as white powder.
Magnesium Glycerophosphate is practically insoluble in ethanol (95).
Magnesium Glycerophosphate dissolves in dilute solutions of acids.
Magnesium Glycerophosphate is hygroscopic.

Identification (1) Mix 1 g of Magnesium Glycerophosphate with 1 g of potassium hydrogen sulfate in a test tube fitted with a glass tube. Heat strongly and direct the white vapor towards a piece of filter paper impregnated with a freshly prepared 1 w/v % sodium pentacyanonitrosylferrate (III) dihydrate solution: the filter paper develops a blue color in contact with piperidine.

(2) Ignite 0.1 g of Magnesium Glycerophosphate in a crucible, add 5 mL of nitric acid to the residue, heat in a water-bath for 1 min and filter. The filtrate responds to the Qualitative Tests (2) for phosphate.

(3) Magnesium Glycerophosphate responds to the Qualitative Tests for magnesium.

Purity (1) ***Clarity and color of solution***—Dissolve 2.5 of Magnesium Glycerophosphate in 50 mL of water: this solution is not more opalescent than the reference suspension

Reference suspension—To 30 mL of standard suspension, add 70 mL of water. Prepare when use and shake well before use.

Standard suspension—Prepare as directed in the reagent under the Test Methods for Plastic Containers.

(2) ***Acid***—Dissolve 1.0 g of Magnesium Glycerophosphate in 100 mL of water and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS): less than 1.5 mL of 0.1 mol/L sodium hydroxide VS consumed.

(3) ***Chloride***—Proceed with 0.20 g Magnesium Glycerophosphate and perform the test. Prepare the control solution with 0.84 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.015 %).

(4) ***Sulfate***—Proceed with 0.20 g of Magnesium Glycerophosphate and perform the test. Prepare the control solution with 0.42 mL of 0.005 mol/L sulfuric acid VS (not more than 0.1 %).

(5) ***Phosphate***—Dissolve 2.5 g of Magnesium Glycerophosphate in 50 mL of water. Pipet 4.0 mL of this solution and add water to make 100 mL. Pipet 1.0 mL of this solution, add water to make 100 mL and use this solution as the test solution. To 100 mL of the test solution, add 4 mL of sulfomolybdic TS and 0.1 mL of tin (II) chloride TS and allow the mixture to stand for 10 minutes: the color from this solution is not more intense than that from a solution containing 2 mL of standard phosphate solution and 98 mL of water (not more than 0.5 %).

(6) ***Heavy metals***—Dissolve 1.0 g of Magnesium Glycerophosphate in 20 mL of water, add 15 mL of hydrochloric acid and 25 mL of 4-methyl-2-pentanone, and mix by shaking for 1 minute. After standing, take the aqueous layer and evaporate to dryness. Dissolve the residue in 2 mL of dilute acetic acid, add 50 mL of water and perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(7) ***Iron***—Place 67 mg of Magnesium Glycerophosphate to a Nessler tube, add water to make 10 mL, add 2 mL of 20 w/v % citric acid solution and 0.1 mL of mercaptoacetic acid to mix, make the solution alkaline by the addition of 10 mol/L ammonia water, add water to make 20 mL and allow this solution to stand for 5 minutes: the color from this solution is not more intense than that from diluted standard iron solution (1 in 10) (not more than 150 ppm).

(8) ***Glycerol and alcohol-soluble substances***—To 1.0 g of Magnesium Glycerophosphate, add 25 ml of ethanol (95), mix by shaking for 2 min, filter and wash the residue with 5 ml of ethanol (95). Combine the filtrate and the washings, evaporate to dryness in a water-bath, dry the residue at 70 °C for 1 h and weigh (not more than 1.5 %).

Loss on Drying Not more than 12.0 % (1 g, 150 °C, 4 hours).

Assay Weigh accurately 0.2 g of Magnesium Glycerophosphate, add 40 mL of water to dissolve, add 10 mL of ammonia-ammonium chloride buffer solution, pH 10 and titrate with 0.1 mol/L disodium ethylenediaminetetraacetate VS (indicator: 50 mg of eriochrome black T-sodium chloride TS) until the color of the solution changes from purple to blue.

Each mL of 0.1 mol/L
disodium ethylenediaminetetraacetate VS
= 2.431 mg of Mg

Containers and Storage ***Containers***—Tight containers.

Magnesium Hydroxide

$Mg(OH)_2$: 58.32

Magnesium dihydroxide [*1309-42-8*]

Magnesium Hydroxide, when dried, contains not less than 95.0 % and not more than 100.5 % of magnesium hydroxide [$Mg(OH)_2$].

Description Magnesium Hydroxide is a white bulky powder.
Magnesium Hydroxide is practically insoluble in water or ethanol (95).

Magnesium Hydroxide is soluble in dilute hydrochloric acid.

Identification A 3 mol/L solution of Magnesium Hydroxide in hydrochloric acid TS (1 in 20) responds to the Qualitative Tests (1) for magnesium.

Purity (1) ***Soluble salts***—Boil 2.0 g of Magnesium Hydroxide with 100 mL of water for 5 minutes in a covered beaker, filter while hot, cool, and dilute the filtrate with water to 100 mL. Titrate 50 mL of the diluted filtrate with 0.1 mol/L sulfuric acid, using methyl red TS as the indicator: not more than 2.0 mL of acid is consumed. Evaporate 25 mL of the diluted filtrate to dryness, and dry at 105 °C for 3 hours: not more than 10 mg of residue remains.

(2) ***Carbonate***—Boil a mixture of 0.10 g of Magnesium Hydroxide with 5 mL of water, cool, and add 5 mL of 6 mol/L acetic acid: not more than a slight effervescence is observed.

(3) ***Calcium***—Transfer 0.25 g of Magnesium Hydroxide, previously dried, to a beaker, add 30 mL of dilute hydrochloric acid (1 in 10), and stir until dissolved, heating if necessary. Transfer this solution to a 200 mL volumetric flask containing 4 mL of lanthanum TS, dilute with water to volume, mix, and use this solution as the test solution. Separately, Weigh accurately 249.7 mg of calcium chloride, previously dried at 300 °C for 3 hours and cooled in a silica gel desiccator for 2 hours, dissolve in small quantities of hydrochloric acid, dilute with water to exactly 100 mL. Pipet each 5.0, 10.0, 15.0 mL of this solution, put each in 1000 mL volumetric flasks, add each 20 mL of Lanthanum TS and 40 mL of diluted hydrochloric acid (1 in 10), dilute with water to volume and use these solutions as the standard solutions. 1 mL of each standard solution contains 5.0, 10.0, 15.0 μg of calcium, respectively. Transfer 4 mL of Lanthanum TS and 10 mL of diluted hydrochloric acid (1 in 10) in a 200 mL volumetric flask and dilute with water to volume and use this solution as the balnk solution. Perform the test with the blank, the test, and the standard solutions as directed under the Atomic Absorption Spectrophotometry according to the following conditions and calculate the calcium content of the test solution from the calibration curve obtained from the absorbance of the standard solution (not more than 1.5 %).

Gas: Dissolved acetylene – Air.
Lamp: A calcium hollow cathode lamp.
Wavelength: 422.7 nm.

(4) ***Heavy metals***—Dissolve 1.0 g of Magnesium Hydroxide in 15 mL of 3 mol/L hydrochloric acid TS, and evaporate the solution on a steam bath to dryness. Dissolve the residue in 20 mL of water, and filter. To the filtrate, add 2 mL of 1 mol/L acetic acid, which should be neutral to litmus, dilute with water to 50 mL and use this solution as test solution. Separately, prepare the control solution with 15 mL of 3 mol/L hydrochloric acid in the same manner as the test solution and 2.0 mL of standard lead solution (not more than 20 ppm) directed for the preparation of the control solution.

(5) ***Lead***—Dissolve 1.0 g of Magnesium Hydroxide in 20 mL of 3 mol/L hydrochloric acid TS and use this solution as the test solution. Transfer the test solution into a separatory funnel, wash with 10 mL of water, and combine the washings into the separatory funnel. Add 6 mL of a solution of diammonium hydrogen citrate and 2 mL of hydroxylamine hydrochloride TS and 2 drops of phenolred, and alkalinize with strong ammonia water. Allow to cool if necessary, add 2 mL of potassium cyanide solution, extract with each 5 mL of dithizone solution for extraction until the extract become green, and combine the extracts in a separatory funnel. To the combined extracts add 20 mL of diluted nitric acid (1 in 100), shake for 30 second to mix, and discard the chloroform layer. To the nitric acid layer add 5.0 mL of standard dithizone solution and 4 mL of ammonia–cyanide TS, shake for 30 second, and compare the color of the solution with that of the control solution obtained from the same procedure with 1.5 mL of diluted standard lead solution (1 in 10): the color obtained from the test solution is not darker than that from the control solution (not more than 1.5 ppm).

Ammonia–cyanide TS—Dissolve 2 g of potassium cyanide in 15 mL of ammonia solution (28) and dilute with water to 100 mL.

(6) ***Arsenic***—To 0.5 g of Magnesium Hydroxide add 10 mL of dilute hydrochloric acid, and warm to dissolve. After cooling, use this solution as the test solution and perform the test (not more than 4 ppm).

Loss on Drying Not more than 2.0 % (1 g, 105 °C, 2 hours).

Loss on Ignition 30.0 ~ 33.0 % (1 g, 800 °C).

Microbial Limit *Escherichia coli* is not observed.

Assay Weigh accurately about 75 mg of Magnesium Hydroxide, previously dried, and transfer to a conical flask. Add 2 mL of 3 mol/L hydrochloride TS and swirl to dissolve. Add 100 mL of water and adjust the reaction of the solution to a pH of 7 with 1 mol/L sodium hydroxide TS, add 5 mL of ammonia·ammonium chloride buffer solution, pH 10.7, and titrate with 0.05 mol/L disodium ethylenediaminetetraacetate VS until the color of the solution changes to blue (indicator : 0.15 mL of eriochrome black T TS)

Each mL of 0.05 mol/L
disodium ethylenediaminetetraacetate VS
= 2.916 mg $Mg(OH)_2$

Containers and Storage ***Containers***—Tight containers.

Magnesium Oxide

MgO: 40.30

[*1309-48-4*]

Magnesium Oxide, when ignited, contains not less than 96.0 % and not more than 101.0 % of magnesium oxide (MgO). When 5 g of Magnesium Oxide has a volume of not more than 30 mL, it may be labeled "Heavy Magnesium Oxide".

Description Magnesium Oxide appears as a white powder or granules and is odorless.
Magnesium Oxide is practically insoluble in water, in ethanol (95) or in ether.
Magnesium Oxide dissolves in dilute hydrochloric acid.
Magnesium Oxide absorbs moisture and carbon dioxide in air.

Identification A solution of Magnesium Oxide in dilute hydrochloric acid (1 in 50) responds to the Qualitative Tests for magnesium salt.

Purity (1) ***Alkali and soluble salts***—Transfer 2.0 g of Magnesium Oxide to a beaker, add 100 mL of water, cover the beaker with a watch-glass, heat in a water-bath for 5 minutes and filter immediately. After cooling, to 50 mL of the filtrate, add 2 drops of methyl red TS and 2.0 mL of 0.05 mol/L sulfuric acid VS: a red color develops. Evaporate 25 mL of the remaining filtrate to dryness and dry the residue at 105 °C for 1 hour: the residue is not more than 10 mg.

(2) ***Carbonate***—Boil 0.10 g of Magnesium Oxide with 5 mL of water, cool and add 5 mL of acetic acid (31): almost no effervescence occurs.

(3) ***Heavy metals***—Dissolve 1.0 g of Magnesium Oxide in 20 mL of dilute hydrochloric acid and evaporate on a water-bath to dryness. Dissolve the residue in 35 mL of water, add 1 drop of phenolphthalein TS, neutralize with ammonia TS, add 2 mL of dilute acetic acid and filter, if necessary. Wash the filter paper with water, add water to the combined washing and the filtrate to make 50 mL and perform the test. Prepare the control solution as follows: to 20 mL of dilute hydrochloric acid, add 1 drop of phenolphthalein TS, neutralize with ammonia TS and add 2 mL of dilute acetic acid, 2.0 mL of standard lead solution and water to make 50 mL (not more than 20 ppm).

(4) ***Iron***—Prepare the test solution with 40 mg of Magnesium Oxide according to Method 1 and perform the test according to Method A. Prepare the control solution with 2.0 mL of standard iron solution (not more than 500 ppm).

(5) ***Calcium oxide***—Weigh accurately 0.25 g of Magnesium Oxide, previously ignited, dissolve in 6 mL of dilute hydrochloric acid by heating. Cool, add 300 mL of water and 3 mL of a solution of L-tartaric acid (1 in 5), then add 10 mL of a solution of 2,2',2''-nitrilotriethanol (3 in 10) and 10 mL of 8 mol/L potassium hydroxide TS, allow to stand for 5 minutes and titrate with 0.01 mol/L disodium ethylenediaminetetraacetate VS until the color of the solution changes from red-purple to blue (indicator: 0.1 g of NN indicator). Perform a blank determination and make any necessary correction,
The mass of calcium oxide (CaO: 56.08) is not more than 1.5 %.

Each mL of 0.01 mol/L
disodium ethylenediaminetetraacetate VS
= 0.5608 mg of CaO

(6) ***Arsenic***—Dissolve 0.5 g of Magnesium Oxide in 5 mL of dilute hydrochloric acid and perform the test with this solution as the test solution (not more than 4 ppm).

(7) ***Acid-insoluble substances***—Mix 2.0 g of Magnesium Oxide with 75 mL of water, add 12 mL of hydrochloric acid drop-wise, while shaking and boil for 5 minutes. Collect the insoluble residue using filter paper for Assay, wash well with water until the last washing shows no turbidity with silver nitrate TS and ignite the residue together with the filter paper: the residue is not more than 2.0 mg.

(8) ***Fluoride***—Perform the test under the conditions of Purity (7) of Natural Aluminium Silicate. The amount of fluoride (F: 19.00) is not more than 0.08 %.

Loss on Ignition Not more than 10.0 % (0.25 g, 900 °C, constant mass).

Assay Ignite Magnesium Oxide to constant mass at 900 °C, weigh accurately about 0.2 g of the residue, dissolve in 10 mL of water to make exactly 100 mL. Pipet 25 mL of this solution, add 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7 and titrate with 0.05 mol/L disodium ethylenediaminetetraacetate VS (indicator: 40 mg of eriochrome black T-sodium chloride indicator). Perform a blank determination and make any necessary correction. From the volume of 0.05 mol/L disodium ethylenediaminetetraacetate VS consumed, deduct the volume of 0.05 mol/L disodium ethylenediaminetetraacetate VS corresponding to the content of calcium oxide(CaO) obtained in the Purity(5).

Each mL of 0.05 mol/L
disodium Ethylenediaminetetraacetate VS
= 2.0152 mg of MgO

Each mL of calcium oxide(CaO) = 0.36 mL of
0.05 mol/L disodium ethylenediaminetetraacetate VS

Containers and Storage ***Containers***—Tight containers.

Magnesium Silicate

Magnesium Silicate contains not less than 45.0 % of silicon dioxide (SiO_2: 60.08) and not less than 20.0 % of magnesium oxide (MgO: 40.30) and the ratio of percentage (%) of magnesium oxide to silicon dioxide is not less than 2.2 and not more than 2.5.

Description Magnesium Silicate is a white, fine powder, is odorless and tasteless.
Magnesium Silicate is practically insoluble in water, in ethanol (95) or in ether.

Identification (1) Mix 0.5 g of Magnesium Silicate with 10 mL of dilute hydrochloric acid, filter and neutralize the filtrate with ammonia TS: the solution responds to the Qualitative Tests for magnesium salt.

(2) Prepare a bead by fusing dibasic sodium ammonium phosphate tetrahydrate on a platinum loop. Place the bead in contact with Magnesium Silicate and fuse again: an infusible matter appears in the bead, which changes to an opaque bead with a web-like structure upon cooling.

Purity (1) ***Soluble salts***—Add 150 mL of water to 10.0 g of Magnesium Silicate, heat on a water-bath for 60 minutes with occasional shakings, then cool, dilute with water to make 150 mL and centrifuge. Dilute 75 mL of the resultant transparent liquid with water to make 100 mL and use this solution as the test solution. Evaporate 25 mL of the test solution on a water-bath to dryness and ignite the residue at 700 °C for 2 hours: the residue is not more than 20 mg.

(2) ***Alkali***—Take 20 mL of the test solution obtained in (1) and add 2 drops of phenolphthalein TS and 1.0 mL of 0.1 mol/L hydrochloric acid VS: no color is observed.

(3) ***Chloride***—Take 10 mL of the test solution obtained in (1), add 6 mL of dilute nitric acid, dilute with water to make 50 mL and perform the test. Prepare the control solution with 0.75 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.053 %).

(4) ***Fluoride***—Weigh 5.0 g of Magnesium Silicate, add 45 mL of 0.1 mol/L hydrochloric acid TS, shake for 15 minutes at room temperature and filter through a membrane filter with pore size of 0.45μm. Wash the used filter with 1 mL of 0.1 mol/L hydrochloric acid TS, repeat 5 times and collect the washings in a flask. Add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL and use this solution as the test solution. Separately, dissolve alizarin complexone in 60 % 2-propanol to contain 0.1 g per mL, filter if necessary and use as the indicator. Pipet 5.0 mL of the test solution into a 25 mL volumetric flask, add 5.0 mL of the indicator, add water to make 25 mL and allow to stand at room temperature for 1 hour. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry, using a solution prepared by mixing 5.0 mL of 0.1 mol/L hydrochloric acid TS, 5.0 mL of the indicator and 15.0 mL of water, as the blank. Determine the absorbances at 620 nm: the absorbance of the test solution is not greater than the absorbance of 5 mL of the following control solution (not more than 10 ppm).

Control solution—Dissolve sodium fluoride in 0.1 mol/L hydrochloric acid TS to contain 2.21 μg per mL.

(5) ***Sulfate***—To residue obtained in (1), add 3 mL of hydrochloric acid and heat on a water-bath for 10 minutes. Add 30 mL of water, combine the washing with the filtrate and dilute with water to make 50 mL with water. To 4 mL of the solution, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.480 %).

(6) ***Heavy metals***—To 1.0 g of Magnesium Silicate, add 20 mL of water and 3 mL of hydrochloric acid and boil for 2 minutes. Filter and wash the residue on the filter with two 5-mL volumes of water. Evaporate the combined filtrate and washing on a water-bath to dryness, add 2 mL of dilute acetic acid to the residue, warm until it dissolves, filter, if necessary, add water to make 50 mL and perform the test. Prepare the control solution with 3.0 mL of standard lead solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(7) ***Arsenic***—To 0.4 g of Magnesium Silicate, add 5 mL of dilute hydrochloric acid, heat gently to boil while shaking well, cool rapidly and centrifuge. Mix the residue with 5 mL of dilute hydrochloric acid with shaking, centrifuge, then add 10 mL of water to the residue and repeat the extraction in the same manner. Concentrate the combined extracts in a water-bath to 5 mL, use this solution as the test solution and perform the test (not more than 5 ppm).

Loss on Ignition Not more than 34.0 % (0.5 g, 850 °C, 3 hours).

Test for Acid-Neutralizing Capacity Weigh accurately about 0.2 g of Magnesium Silicate and transfer in a glass-stoppered flask. Add exactly 30 mL of 0.1 mol/L hydrochloric acid VS and 20 mL of water, shake at 37 ± 2 °C for 1 hour, and cool. Pipet 25 mL of the clear supernatant liquid, and titrate the excess hydrochloric acid, while stirring well, with 0.1 mol/L sodium hydroxide VS until the pH becomes 3.5. 1 g of Magnesium Silicate, calculated on the anhydrous basis by making allowance for the observed loss on ignition determined as directed under the Loss on Ignition, consumes not less than 140 mL and not more than 160 mL of 0.1 mol/L hydrochloric acid.VS.

Assay (1) ***Silicon dioxide***—Weigh accurately about 0.7 g of Magnesium Silicate, add 10 mL of 0.5 mol/L sulfuric acid TS, evaporate on a water-bath to dryness, add 25 mL of water to the residue and heat on a water-

bath for 15 minutes with occasional stirring. Filter the clear supernatant liquid through filter paper for Assay, add 25 mL of hot water to the residue, stir and decant the clear supernatant liquid on the filter paper to filter. Wash the residue in the same manner with two 25 mL volumes of hot water, transfer the residue onto the filter paper and wash with hot water until the last washing does not respond to the Qualitative Tests (1) for sulfate. Place the residue and the filter paper in a platinum crucible, incinerate with strong heating and ignite between 775 °C and 825 °C for 30 minutes, then cool and weigh the residue as *a* (g). Moisten the residue with water and add 6 mL of hydrofluoric acid and 3 drops of sulfuric acid. Evaporate to dryness, ignite for 5 minutes, cool and weigh the residue as *b* (g).

$$\text{Content (\%) of silicon dioxide } (SiO_2) = \frac{a - b}{\text{amount (g) of the sample}} \times 100$$

(2) ***Magnesium oxide***—Weigh accurately about 0.3 g of Magnesium Silicate, transfer to an Erlenmeyer flask, add 10 mL of 0.5 mol/L sulfuric acid TS and heat on a water-bath for 15 minutes. Cool, transfer to a volumetric flask, wash the Erlenmeyer flask with water, add the washing to the volumetric flask, dilute with water to make 100 mL and filter. Pipet 50.0 mL of the filtrate, shake with 50 mL of water and 5 mL of diluted 2,2',2"-nitrile triethanol (1 in 2), add 2.0 mL of ammonia TS and 10 mL of ammonia-ammonium chloride buffer solution, pH 10.7 and titrate with 0.05 mol/L disodium ethylenediaminetetraacetate VS (indicator: 40 mg of eriochrome black T-sodium chloride indicator).

Each mL of 0.05 mol/L
disodium ethylenediaminetetraacetate VS
= 2.0152 mg of MgO

(3) ***Ratio of percentages (%) of magnesium oxide (MgO) to silicon dioxide (SiO_2)***—Calculate the quotient from the percentages obtained in (1) and (2).

Containers and Storage ***Containers***—Well-closed containers.

Magnesium Sulfate Hydrate

$MgSO_4 \cdot 7H_2O$: 246.48

Magnesium sulfate hetahydrate [*10034-99-8*]

Magnesium Sulfate Hydrate, when ignited, contains not less than 99.0 % and not more than 101.0 % of magnesium sulfate ($MgSO_4$: 120.37).

Description Magnesium Sulfate Hydrate appears as colorless or white crystals, has a cooling, saline and bitter taste.

Magnesium Sulfate Hydrate is very soluble in water and practically insoluble in ethanol (95) or in ether.

Identification A solution of Magnesium Sulfate Hydrate (1 in 40) responds to the Qualitative Tests for magnesium salt and for sulfate.

pH Dissolve 1.0 g of Magnesium Sulfate Hydrate in 20 mL of water: the pH of this solution is between 5.0 and 8.2.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Magnesium Sulfate Hydrate in 20 mL of water: the solution is clear and colorless.

(2) ***Chloride***—Perform the test with 1.0 g of Magnesium Sulfate Hydrate. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014 %).

(3) ***Heavy metals***—Proceed with 2.0 g of Magnesium Sulfate Hydrate according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(4) ***Zinc***—Dissolve 2.0 g of Magnesium Sulfate Hydrate in 20 mL of water and add 1 mL of acetic acid (31) and 5 drops of potassium hexacyanoferrate (II) TS: no turbidity is produced.

(5) ***Iron***—(i) Perform this test when Magnesium Sulfate Hydrate is used in an oral preparation. Weigh accurately 0.5 g of Magnesium Sulfate Hydrate, dissolve in water to make 40 mL, dilute with water to make 45 mL if necessary, add 2 mL of hydrochloric acid, mix, and use this solution as the test solution. To 1.0 mL of standard iron solution add water to make 45 mL, add 2 mL of hydrochloric acid, and use this solution as the standard solution. To the test solution and standard solution add 50 mg of ammonium peroxydisulfate and 3 mL of 30 % ammonium thiocyanate solution, and mix: the color obtained from the test solution is not more intense than that from the standard solution (not more than 20 ppm).

(ii) Perform this test when Magnesium Sulfate Hydrate is used in a parenteral preparation. Rinse all glass vessels used in this test with diluted hydrochloric acid before use. Weigh accurately 10.0 g of Magnesium Sulfate Hydrate, add 35 mL of a solution of hydrochloric acid (1 in 1000), sonicate to dissolve, and use this solution as the test solution. Pipet 5.0 mL of standard iron solution, add a solution of hydrochloric acid (1 in 1000) to make exactly 50 mL, and use this solution as the iron standard solution. Separately, to three 50 mL volumetric flasks transfer 2.0, 5.0, and 10.0 mL of the iron standard solution, add a solution of hydrochloric acid (1 in 1000) to make 35 mL so that the solutions contain 2.0, 5.0, and 10.0 μg of iron. Separately, to a 50 mL volumetric flask transfer 35 mL of a diluted solution of hydrochloric acid (1 in 1000), and use this solution as the blank solution. To the test solutions, standard solutions, and blank solution add 5 mL of ascorbic acid TS and 5 mL of the color reagent, add a solution of hydrochloric acid (1 in 1000) to make 50 mL, allow

to stand for 10 minutes, and perform the test with the test solutions and standard solutions as directed under Ultraviolet-visible Spectrophotometry, using the blank solution as the blank. Determine the absorbances at about 594 nm, and calculate the content of iron in the test solution using the calibration curve obtained from the absorbances of the standard solutions: not more than 0.5 ppm.

Ascorbic acid TS—Dissolve 1.34 g of L-ascorbic acid in water to make 100 mL. Prepare this solution before use.

Coloring agent—Dissolve 0.38 g of 3-(2-pyridyl)-5,6-di-(2-furyl)-1,2,4-triazine-5'5"-disulfonic acid, disodium salt in a solution of ammonium acetate (1 in 10) to make 100 mL. Prepare this solution before use.

(6) ***Calcium***—Dissolve 1.0 g of Magnesium Sulfate Hydrate in 5.0 mL of dilute hydrochloric acid, add water to make 100 mL and use this solution as the test solution. Separately, dissolve 1.0 g of Magnesium Sulfate Hydrate in 2.0 mL of standard calcium solution and 5.0 mL of dilute hydrochloric acid, add water to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Atomic Absorption Spectrophotometry according to the following conditions and determine the absorbances, A_T and A_S, respectively: A_T is not more than $A_S - A_T$ (not more than 0.02 %)

Gas: Dissolved acetylene or hydrogen – Air
Lamp: Calcium hollow-cathode lamp
Wavelength: 422.7 nm

(7) ***Arsenic***—Prepare the test solution with 0.1 g of Magnesium Sulfate according to Method 1 and perform the test (not more than 2 ppm).

(8) ***Selenium***—Proceed with 0.2 g of Magnesium Sulfate Hydrate, accurately weighed, as directed under Oxygen Flask Combustion Method, using 50 mL of 0.25 mol/L nitric acid TS as the absorbing liquid. Use a 1000 mL combustion flask. After combustion, wash the stopper and the inside of the flask with 10 mL of water. Transfer the liquid inside the flask to a 150 mL beaker, using 20 mL of water. Heat gently to boil, boil for 10 minutes, cool to room temperature, and use this solution as the test solution. Separately, pipet 6.0 mL of selenium standard stock solution, add 25 mL of diluted nitric acid (1 in 30) and 25 mL of water, and use this solution as the standard solution. Adjust the pH of the test solution and standard solution to 2.0 ± 0.2 with diluted ammonia solution (28) (1 in 2), add water to make exactly 60 mL, transfer to a separatory funnel using 10 mL of water, and wash the separatory funnel with 10 mL of water. Add 0.2 g of hydroxylamine hydrochloride, stir to dissolve, add immediately 5.0 mL of 2,3-diaminonaphthalene TS, stopper, stir to mix, and allow to stand at room temperature for 100 minutes. Add 5.0 mL of cyclohexane, shake vigorously for 2 minutes, allow the layers to separate, discard the water layer, centrifuge the cyclohexane extract to remove water, and take the cyclohexane layer. Determine the absorbances at 380 nm of these solutions as directed under Ultraviolet-visible Spectrophotometry, using a solution prepared by adding 25 mL of water to 25 mL of diluted nitric acid (1 in 30) and proceeding in the same manner, as the blank: the absorbance obtained from the test solution is not more than that from the standard solution (not more than 30 ppm).

Loss on Ignition 45.0 ~ 52.0 % (1 g, after drying at 105 ºC for 2 hours, ignite at 450 ºC for 3 hours).

Assay Weigh accurately about 0.6 g of Magnesium Sulfate, previously ignited at 450 °C for 3 hours after drying at 105 °C for 2 hours and dissolve in 2 mL of dilute hydrochloric acid and water to make exactly 100 mL. Pipet 25 mL of this solution and perform the test as directed in the Assay under Magnesium Oxide.

Each mL of 0.05 mol/L
disodium ethylenediaminetetraacetate VS
= 6.018 mg of $MgSO_4$

Containers and Storage ***Containers***—Well-closed containers.

Magnesium Sulfate Injection

Magnesium Sulfate Injection is an aqueous solution for injection. Magnesium Sulfate Injection contains not less than 95.0 % and not more than 105.0 % of the labeled amount of magnesium sulfate hydrate ($MgSO_4 \cdot 7H_2O$: 246.48).

Method of Preparation Prepare as directed under Injections, with Magnesium Sulfate Hydrate.

Description Magnesium Sulfate Injection is a clear and colorless liquid.

Identification Measure a volume of Magnesium Sulfate Injection, equivalent to 0.5 g of Magnesium Sulfate Hydrate according to the labeled amount and add water to make 20 mL: the solution responds to the Qualitative Tests for magnesium salt and for sulfate.

pH 5.5 ~ 7.0. When the labeled concentration excceeds 5 w/v %, prepare a solution of 5 w/v % with water, and perform the test.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.90 EU/mg of Magnesium Sulfate Hydrate. Perform the test after diluting with water for the test of endotoxin to 5 w/v %.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

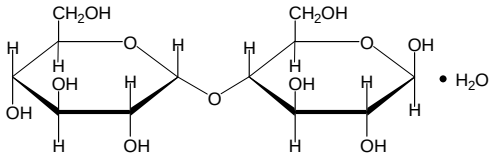
Determination of Volume of Injection in Containers It meets the requirement.

Assay Pipet a volume of Magnesium Sulfate Injection, equivalent to about 0.3 g of magnesium sulfate Hydrate ($MgSO_4 \cdot 7H_2O$), add water to make 75 mL, and add 5 mL of pH 10.7 ammonia-ammonium chloride buffer solution. Proceed as directed in the Assay under Magnesium Oxide.

Each mL of 0.05 mol/L
disodium ethylenediaminetetraacetate VS
= 12.32 mg of $MgSO_4 \cdot 7H_2O$

Containers and Storage ***Containers***—Hermetic containers. Plastic containers for aqueous injections may be used.

Maltose Hydrate



maltose $C_{12}H_{22}O_{11} \cdot H_2O$: 360.31

(2*R*,3*S*,4*S*,5*R*,6*R*)-2-(Hydroxymethyl)-6-[(2*R*,3*S*,4*R*,5*R*)-4,5,6-trihydroxy-2-(hydroxymethyl)oxan-3-yl]oxyoxane-3,4,5-triol hydrate [*6363-53-7*]

Maltose Hydrate, when dried, contains not less than 98.0 % and not more than 101.0 % of maltose hydrate ($C_{12}H_{22}O_{11} \cdot H_2O$).

Description Maltose Hydrate appears as white, crystals or crystalline powder and has a sweet taste.
Maltose Hydrate is freely soluble in water, very slightly soluble in ethanol (95) and practically insoluble in ether.

Identification (1) Dissolve 0.5 g of Maltose Hydrate in 5 mL of water, add 5 mL of ammonia TS and heat for 5 minutes in a water-bath: an orange color is observed.

(2) Add 2 to 3 drops of a solution of Maltose Hydrate (1 in 50) to 5 mL of boiling Fehling TS: a red precipitate is produced.

Specific Optical Rotation $[\alpha]_D^{20}$: +126 ~ +131°.
Weigh accurately about 10 g of Maltose Hydrate, previously dried, dissolve in 0.2 mL of ammonia TS and water to make exactly 100 mL and determine the specific rotation of this solution in a 100-mm cell.

pH The pH of a solution of Maltose Hydrate (1 in 10) is between 4.5 and 6.5.

Purity (1) ***Clarity and color of solution***—Put 10 g of Maltose Hydrate in 30 mL of water in a Nessler tube, warm at 60 °C on a water-bath to dissolve, cool and add water to make 50 mL: the solution is clear and has no more color than the following control solution.

Control solution—Add water to a mixture of 1.0 mL of cobalt (II) chloride hexahydrate stock CS, 3.0 mL of ferric chloride stock CS and 2.0 mL of iron (III) chloride hexahydrate stock CS to make 10.0 mL. To 1.0 mL of this solution, add water to make 50 mL.

(2) ***Chloride***—Perform the test with 2.0 g of Maltose Hydrate. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018 %).

(3) ***Sulfate***—Perform the test with 2.0 g of Maltose Hydrate. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024 %).

(4) ***Heavy metals***—Proceed with 5.0 g of Maltose Hydrate according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 4 ppm).

(5) ***Arsenic***—Dissolve 1.5 g of Maltose Hydrate in 5 mL of water, add 5 mL of dilute sulfuric acid and 1 mL of bromine TS, heat on a water-bath for 5 minutes, then heat to concentrate to 5 mL and use this solution as the test solution after cooling. Perform the test (not more than 1.3 ppm).

(6) ***Dextrin, soluble starch and sulfite***—Dissolve 1.0 g of Maltose Hydrate in 10 mL of water and add 1 drop of iodine TS: a yellow color appears and the color changes to a blue by adding 1 drop of starch TS.

(7) ***Nitrogen***—Weigh accurately about 2.0 g of Maltose Hydrate and perform the test as directed under the Nitrogen Determination using 10 mL of sulfuric acid for the decomposition and 45 mL of a solution of sodium hydroxide (2 in 5) for the addition: the amount of nitrogen (N: 14.01) is not more than 0.01 %.

(8) ***Related substances***—Dissolve 0.5 g of Maltose Hydrate in 10 mL of water and use this solution as the test solution. Pipet 1 mL of the test solution, add water to make exactly 100 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine the peak areas from the test solution and the standard solution by the automatic integration method: the total area of the peaks which appear before the peak of Maltose Hydrate from the test solution is not larger than 1.5 times of the peak area of Maltose Hydrate from the standard

solution. The total area of the peaks which appear after the peaks of Maltose Hydrate from the test solution is not larger than 0.5 times of the peak area of Maltose Hydrate from the standard solution.

Operating conditions

Detector, column, temperature, mobile phase, flow rate and system performance: Proceed as directed in the Assay.

Detection sensitivity: Adjust the sensitivity so that the peak height of maltose obtained from 20 μL of the standard solution is about 30 mm.

Time span of measurement: About 2 times as long as the retention time of Maltose Hydrate.

Loss on Drying Not more than 0.5 % (1 g, 80 °C, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.1 g each of Maltose Hydrate and Maltose Hydrate RS, previously dried and dissolve in 10 mL each of the internal standard solution and use these solutions as the test solution and the standard solution, respectively. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the ratios, Q_T and Q_S, of the peak area of maltose to that of the internal standard, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of maltose hydrate } (C_{12}H_{22}O_{11}\cdot H_2O) = \text{Amount (mg) of Maltose Hydrate RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of ethylene glycol (1 in 50).

Operating conditions

Detector: A differential refractometer.

Column: A stainless steel column, about 8 mm in internal diameter and about 55 cm in length, packed with gel-typed strong acid cation-exchange resin for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 50 °C.

Mobile phase: Water.

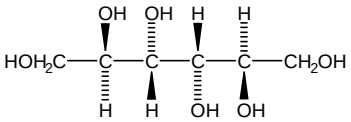
Flow rate: Adjust the flow rate so that the retention time of maltose is about 18 minutes.

System suitability

System peformance: Dissolve 0.25 g of maltose, 0.25 g of Glucose and 0.4 g of ethylene glycol in water to make 100 mL. When the procedure is run with 20 μL of this solution according to the above operating conditions, maltose, glucose and ethylene glycol are eluted in this order with the resolution of between the peaks of maltose and glucose being not less than 4.

Containers and Storage *Containers*—Tight containers.

D-Mannitol



$C_6H_{14}O_6$: 182.17

(2*R*,3*R*,4*R*,5*R*)-Hexane-1,2,3,4,5,6-hexol [*69-65-8*]

D-Mannitol, when dried, contains not less than 98.0 % and not more than 101.0 % of D-mannitol ($C_6H_{14}O_6$).

Description D-Mannitol is a white, crystalline powder, is odorless and has a sweet taste with a cold sensation.

D-Mannitol is freely soluble in water and practically insoluble in ethanol (95) or in ether.

D-Mannitol dissolves in sodium hydroxide TS.

Identification (1) Take 5 drops of a saturated solution of D-Mannitol and add 1 mL of iron (III) chloride TS and 5 drops of a solution of sodium hydroxide (1 in 5): a yellow precipitate is produced. Shake this solution vigorously: a clear solution is produced. On addition of a solution of sodium hydroxide (1 in 5), no precipitate is produced.

(2) Determine the infrared spectra of D-Mannitol and D-Mannitol RS as directed in the potassium Bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears between the spectra, dissolve 1 g of D-Mannitol in 3 mL of warm water, then allow to stand at 5 °C for 24 hours or until crystals appear, and filter. Wash the crystals so obtained with a few amount of cold water, dry at 105 °C for 4 hours, and perform the test with the crystals.

Specific Optical Rotation $[\alpha]_D^{20}$: +137 ~ +145°. Weigh accurately 1.0 g of D-Mannitol, previously dried, dissolve in 80 mL of a solution of hexaammonium heptamolybdate tetrahydrate (1 in 20) and add diluted sulfuric acid (1 in 35) to make exactly 100 mL. Measure the optical rotation of this solution in a 100-mm cell.

Melting Point 166 ~ 169 °C.

Purity (1) ***Clarity and color of solution***—Dissolve 2.0 g of D-Mannitol in 10 mL of water by warming: the solution is clear and colorless.

(2) ***Acid***—Dissolve 5.0 g of D-Mannitol in 50 mL of freshly boiled and cooled water and add 1 drop of phenolphthalein TS and 0.5 mL of 0.01 mol/L sodium

hydroxide VS: a red color is observed.

(3) ***Chloride***—Perform the test with 2.0 g of D-Mannitol. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.007 %).

(4) ***Sulfate***—Perform the test with 2.0 g of D-Mannitol. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010 %).

(5) ***Heavy metals***—Proceed with 5.0 g of D-Mannitol according to Method 1 and perform the test. Prepare the control solution with 2.5 mL of standard lead solution (not more than 5 ppm).

(6) ***Nikel***—Dissolve 0.5 g of D-Mannitol in 5 mL of water, add 3 drops of dimethylglyoxime TS and 3 drops of ammonia TS and allow to stand for 5 minuets: no red color is observed.

(7) ***Arsenic***—Prepare the test solution with 1.5 g of D-Mannitol according to Method 1 and perform the test (not more than 1.3 ppm).

(8) ***Sugars***—Take 5.0 g of D-Mannitol, add 15 mL of water and 4.0 mL of dilute hydrochloric acid and heat under a reflux condenser on a water-bath for 3 hours. After cooling, neutralize with sodium hydroxide TS (indicator: 2 drops of methyl orange TS) and add water to make exactly 50 mL. Pipet 10 mL of this solution into a flask, boil gently with 10 mL of water and 40 mL of Fehling' TS for 3 minutes and allow to stand to precipitate cuprous oxide. Filter the clear supernatant liquid through a glass filter (G4), wash the precipitate with hot water until the last washing no longer shows an alkaline reaction and filter the washings through the glass filter described above. Dissolve the precipitate in 20 mL of iron (III) sulfate TS in the flask filter through the glass filter described above and wash the filter with water. Combine the washings and the filtrate, heat to 80 °C and titrate with 0.02 mol/L potassium permanganate VS: the consumed volume is not more than 1.0 mL.

Loss on Drying Not more than 0.3 % (1 g, 105 °C, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.2 g of D-Mannitol, previously dried and dissolve in water to make exactly 100 mL. Pipet 10 mL of the solution into an iodine flask, add exactly 50 mL of potassium periodate TS and heat for 15 minutes in a water-bath. After cooling, add 2.5 g of potassium iodide, stopper tightly and shake well. Allow to stand for 5 minutes in a dark place and titrate the produced iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 1.8217 mg of $C_6H_{14}O_6$

Containers and Storage ***Containers***—Well-closed containers.

D-Mannitol Injection

D-Mannitol Injection is an aqueous solution for injection. D-Mannitol Injection contains not less than 95.0 % and not more than 105.0 % of the labeled amount of D-mannitol ($C_6H_{14}O_6$: 182.17).

Method of Preparation Prepare as directed under Injections, with D-Mannitol. No preservative is added.

Description D-Mannitol Injection is a clear, colorless liquid and has a sweet taste.
D-Mannitol Injection may precipitate crystals.

Identification Concentrate D-Mannitol Injection on a water-bath to make saturated solution. Proceed with 5 drops of this solution as directed in the Identification (1) under D-Mannitol.

pH 4.5 ~ 7.0.

Residue on Ignition Evaporate exactly measured volume of D-Mannitol Injection, equivalent to 1.0 g of D-Mannitol, on a water-bath to dryness and perform the test: the residue is not more than 1.0 mg.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.5 EU/mL of D-Mannitol Injection.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

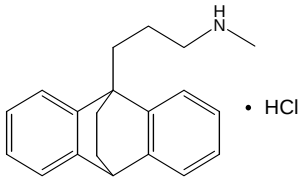
Determination of Volume of Injection in Container It meets the requirement.

Assay Measure exactly a volume of D-Mannitol Injection, equivalent to about 5 g of D-mannitol ($C_6H_{14}O_6$), add water to make exactly 250 mL. To exactly 10 mL of this solution, add water to make exactly 100 mL. Measure exactly 10 mL of this solution into an idodine flask, and proceed as directed in the Assay under D-Mannitol

Each mL of 0.1 mol/L sodium thiosulfate VS
= 1.822 mg of $C_6H_{14}O_6$

Containers and Storage ***Containers***—Hermetic containers.

Maprotiline Hydrochloride



$C_{20}H_{23}N·HCl$: 313.86

N-Methyl-9,10-ethanoanthracene-9(10*H*)-propanamine monohydrochloride [*10347-81-6*]

Maprotiline Hydrochloride, when dried, contains not less than 99.0 % and not more than 101.0 % of maprotiline hydrochloride ($C_{20}H_{23}N·HCl$).

Description Maprotiline Hydrochloride is a white crystalline powder.
Maprotiline Hydrochloride is soluble in methanol or in acetic acid (100), sparingly soluble in ethanol (99.5) and slightly soluble in water.

Melting point—about 244 °C (with decomposition).

Identification (1) Determine the absorption spectra of the solutions of Maprotiline Hydrochloride and Maprotiline Hydrochloride RS in methanol (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Maprotiline Hydrochloride and Maprotiline Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears between the spectra, dissolve Maprotiline Hydrochloride and Maprotiline Hydrochloride RS in ethanol (99.5), evaporate ethanol (99.5) to dryness and perform the test with the residues.

(3) Take 5 mL of a solution of Maprotiline Hydrochloride (1 in 200) add 2 mL of ammonia TS, heat on a water bath for 5 minutes, cool and filter. Acidify the filtrate with dilute nitric acid: the solution responds to Qualitative Test for chloride.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Maprotiline Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Dissolve 0.10 g of Maprotiline Hydrochloride in 5 mL of methanol and use this solution as the test solution. Pipet 1 mL of the test solution, add methanol to make exactly 200 mL and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop with a mixture of 2-butanol, diluted ammonia solution (28) (1 in 3) and ethyl acetate (14 : 5 : 4) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the number of the spot other than the principal spot from the test solution is not more than 2 and they are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

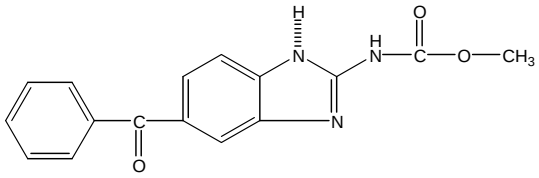
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.25 g of Maprotiline Hydrochloride, previously dried, dissolve in 180 mL of acetic acid (100), add 8 mL of a solution of bismuth nitrate pentahydrate in acetic acid (100) (1 in 50) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Methods in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 31.386 mg of $C_{20}H_{23}N·HCl$

Containers and Storage ***Containers***—Well-closed containers.

Mebendazole



$C_{16}H_{13}N_3O_3$: 295.29

Methyl (5-benzoyl-1*H*-benzimidazol-2-yl)carbamate [*31431-39-7*]

Mebendazole, when dried, contains not less than 98.0 % and not more than 102.0 % of mebendazole ($C_{16}H_{13}N_3O_3$).

Description Mebendazole is a white to pale yellow powder.
Mebendazole is freely soluble in formic acid and practically insoluble in water, in dilute hydrochloric acid, in ethanol (95), in chloroform or in ether.

Melting point—About 290 °C

Identification Determine the infrared spectra of Mebendazole and Mebendazole RS, previously dried, as directed in the potassium bromide disk method un-

der Infrared Spectrophotometry: both spectra exhibit the similar intensities of absorption at the same wavenumbers.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Mebendazole according to Method 2 and perform the test. Prepare the control solution with 2.0 mL standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Dissolve 50 mg of Mebendazole in 1.0 mL of 96 % formic acid, add chloroform to make exactly 10 mL and use this solution as the test solution. Weigh accurately Mebendazole RS, prepare to make the solution containing 5 mg per mL similarly as the test solution and use this solution as the standard stock solution. Transfer 1.0 mL of this standard stock solution to a volumetric flask, add a mixture of chloroform and 96.0 % formic acid (9 : 1) to make 200 mL, mix and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, formic acid and methanol (90 : 5 : 5) to a distance of about 15 cm and air-dry. Examine the plate under ultraviolet light (main wavelength: 254 nm): the R_f value of the principal spot from the test solution corresponds to the R_f value of the principal spot from the dilute standard solution. The spot other than the principal spot from the test solution is not larger and not more intense than the spot from the dilute standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.225 g of Mebendazole, dissolve in 30 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.529 mg of $C_{16}H_{13}N_3O_3$

Containers and Storage ***Containers***—Well-closed containers.

Mebendazole Tablets

Mebendazole, when dried, contains not less than 90.0 % and not more than 110.0 % of mebendazole ($C_{16}H_{13}N_3O_3$: 295.29).

Method of Preparation Prepare as directed under Tablets, with Mebendazole.

Identification Finely powder a quantity of Tablets, equivalent to about 200 mg of Mebendazole and mix the powder with 20 mL of a mixture of chloroform and formic acid (19 : 1). Warm the suspension on a water-bath for a few minutes, cool and filter through a glass filter and use this solution as the test solution. Dissolve Mebendazole RS in a mixture of chloroform and formic acid (19 : 1) containing 10 mg per mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography and develop the plate in a mixture of chloroform, formic acid and methanol (90 : 5 : 5) to a distance of about 15 cm and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the R_f value of the principal spot obtained from the test solution corresponds to that obtained from the standard solution.

Dissolution Test Perform the test with 1 tablet of Mebendazole Tablets at 75 revolutions per minute according to the Method 2 under the Dissolution Test, using 900 mL of 0.1 mol/L hydrochloric acid containing 1.0 % sodium lauryl sulfate as the dissolution solution. Take the dissolved solution after 120 minutes from the start of the test and filter. Use this solution as the test solution. Separately, weigh accurately about 25 mg of Mebendazole RS, add 10.0 mL of formic acid and add methanol to make exactly 50 ml. Take this solution, make the same concentration of the test solution and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions.
The dissolution rate of Menbendazole Tablets in 120 minutes is not less than 75 %.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm to 10 μm in particle diameter).

Mobile phase: A mixture of the buffer solution and acetonitrile (7:3)

Buffer solution—Weigh 8.0 g of sodium hydroxide, dissolve in water to make 200 mL. Add 3.0 g of sodium lauryl sulfate and 20 mL of phosphoric acid to adjust the pH of the solution to 2.5.

Flow rate: 1 mL/minute.

System suitability

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of Mebendazole is not more than 2.0 %.

Uniformity of Dosage Units It meets the requirement when the content uniformity test is performed according to the following method.

Mix 1 Tablet with 20 mL of 96 % formic acid in a 100-mL volumetric flask, and heat on a steam bath for 15 minutes. Cool, add 2-propanol to volume, mix, and filter. Transfer an accurately measured portion of the filtrate, equivalent to 1 mg of mebendazole, to a 100-mL volumetric flask, dilute with 2-propanol to volume, and mix. This solution is used as the test solution. Separately, transfer about 20 mg of Mebendazole RS, accurately weighed, to a 10-mL volumetric flask, add 4 mL of 96 % formic acid, and mix to dissolve. Add 2-propanol to volume, and mix. Pipet 0.5 mL of this solution into a 100-mL volumetric flask, dilute with 2-propanol to volume, and mix. This solution is used as the standard solution. As directed under Ultraviolet-visible Spectrophotometry, concomitantly determine the absorbance (A_S) of the standard solution and that (A_T) of the test solution at the wavelength of maximum absorbance at about 310 nm, using diluted formic acid (1 in 500) as the blank.

$$\text{Amount (mg) of mebendazole } (C_{16}H_{13}N_3O_3) \text{ in 1 tablet} = \frac{TC}{D} \times \frac{A_T}{A_S}$$

T: Labeled amount (mg) of mebendazole in the Tablet

C: Concentration (μg/mL) of Mebendazole RS in the standard solution

D: Concentration (μg/mL) of mebendazole in the test solution, based on the labeled amount per Tablet and the extent of dilution

Assay Weigh accurately and powder not less than 20 Mebendazole Tablets. Weigh accurately a portion of the powder, equivalent to about 500 mg of Mebendazole, add 50 mL of formic acid and heat at 50 °C for 15 minutes in a water-bath. Shake for 1 hour with a shaker, add water to make exactly 100 mL and filter. Pipet 5.0 mL of this solution and add a mixture of methanol and formic acid (9:1) to make exactly 100 mL. Pipet 5.0 mL of this solution, add 25 mL of mobile phase, shake well and filter. Use this solution as the test solution. Separately, weigh accurately about 25 mg of Mebendazole RS, add 10 mL of formic acid and warm at 50 °C for 15 minutes in a water-bath. Shake for 5 minutes with a shaker and add methanol to make exactly 100 mL. Pipet 5.0 mL of this solution, add mobile phase to make exactly 25 mL, shake well and filter. Use this solution as the standard solution. Perform the test with 15 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Measure the peak areas, A_T and A_S, of Mebendazole, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of mebendazole } (C_{16}H_{13}N_3O_3) = \text{Amount (mg) of Mebendazole RS} \times \frac{A_T}{A_S} \times \frac{1}{20}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 247 nm).

Column: A stainless steel column, about 3.9 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Column temperature: A constant temperature of about 30 °C.

Mobile phase: Titrate a mixture of methanol and 0.05 mol/L of monobasic potassium phosphate TS (60 : 40) with 0.1 mol/L phosphoric acid TS or 1 mol/L sodium hydroxide TS to pH 5.5.

Flow rate: 1.5 mL/minute.

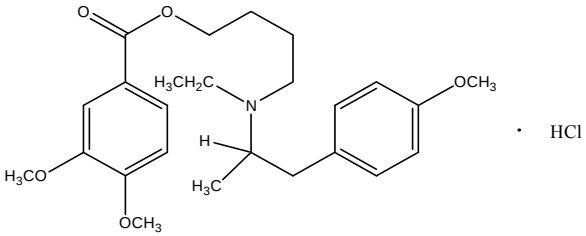
System suitability

System performance: When the procedure is run with 15 μL of the standard solution under the above operating conditions, the symmetry factor of mebendazole peak is not more than 2.0.

System repeatability: When the test is repeated 6 times with 15 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of mebendazole is not more than 1.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Mebeverin Hydrochloride



and enantiomer

$C_{25}H_{35}NO_5 \cdot HCl$: 466.01

(*RS*)-4-[Ethyl-[1-(4-methoxyphenyl)propan-2-yl]amino]butyl-3,4-dimethoxybenzoate hydrochloride [*14664-75-6*]

Mebeverin Hydrochloride contains not less than 99.0 % and not more than 101.0 % of mebeverin hydrochloride ($C_{25}H_{35}NO_5 \cdot HCl$), calculated on the dried basis.

Description Mebeverin Hydrochloride is a white crystalline powder.

Mebeverin Hydrochloride is very soluble in water, freely soluble in ethanol (95), and practically insoluble

in ether.

Identification (1) Determine the infrared spectra of Mebeverin Hydrochloride and Mebeverin Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Dissolve 25 mg of Mebeverin Hydrochloride in 25 mL of water, acidify by the addition of 2 mol/L nitric acid and centrifuge: the supernatant liquid responds to the Qualitative Tests (2) for chloride.

pH Dissolve 2 g of Mebeverin Hydrochloride in 100 mL of water: the pH of this solution is between 4.5 and 6.5.

Purity (1) ***Ether extract***—Dissolve 40 mg of Mebeverin Hydrochloride in 25 mL of 2 mol/L hydrochloric acid TS, add 50 mL of ether, mix by shaking for 1 minute, wash the ether layer three times each with 25 mL of water, evaporate the ether layer to dryness, add a portion of methanol to residue to make 20 mL and determine the absorbance of the solution at 260 nm as directed under Ultraviolet-visible Spectrophotometry using methanol as the reference: the absorbance is not more than 0.23.

(2) ***Non-tertiary amine***—Dissolve 0.5 g of Mebeverin Hydrochloride in 5 mL of pyridine, add 5 mL of cupric chloride-pyridine TS, heat at 50 °C for 30 minutes, cool, add acetone to make 50 mL and use this solution as the test solution. Separately, proceed with a solution of 0.0060 w/v % di-*n*-butylamine in 5 mL of pyridine in a manner similar to the preparation of the test solution and use this solution as the control solution. Prepare a solution with 5 mL of pyridine in a manner similar to the preparation of the test solution and use this solution as the blank solution. Perform the test with the test solution and the control solution as directed under Ultraviolet-visible Spectrophotometry and determine the absorbance of these solutions as 405 nm using the blank solution as the reference: the absorbance of the test solution is not more than that of the control solution.

(3) ***Related substance***—Weigh 20 mg of Mebeverin Hydrochloride, add acetone to make exactly 10 mL and use this solution as the test solution. Separately, weigh 10 mg of Mebeverin Hydrochloride, add acetone to make exactly 100 mL and use this solution as the standard solution (1). Weigh 2.0 mg of veratric acid, add acetone to make exactly 100 mL and use this solution as the standard solution (2). Perform the test with the test solution, the standard solutions (1) and (2) as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution, the standard solution (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), chloroform and ammonia solution (28) (50:50:1) to a distance of about 15 cm and air-dry the plate. Expose the plate under ultraviolet light (main wavelength 254 nm) and allow the plate to stand in iodine vapor for 1 hour: the spot corresponding to the veratric acid from the test solution is not more intense than that from the standard solution (2) and the spots other than the principal spot are not more intense than the principal spot from the test solution (1).

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 1 hour).

Residue on Ignition Not more than 0.1 % (1 g).

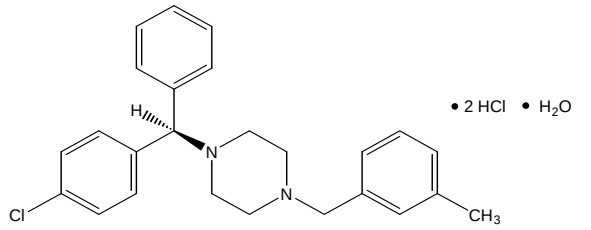
Assay Weigh accurately about 0.4 g of Mebeverin Hydrochloride in 50 mL of acetic acid (100), add 7 mL of mercury (II) acetate TS and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 46.60 mg of $C_{25}H_{35}NO_5 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and not exceeding 30 °C.

Meclizine Hydrochloride Hydrate



and enantiomer

Meclizine Hydrochloride

$C_{25}H_{27}ClN_2 \cdot 2HCl \cdot H_2O$: 481.89

(*RS*)-1-[(4-Chlorophenyl)(phenyl)methyl]-4-(3-methylbenzyl)piperazine hydrate dihydrochloride [*31884-77-2*]

Meclizine Hydrochloride Hydrate contains not less than 97.0 % and not more than 100.5 % of meclizine hydrochloride ($C_{25}H_{27}ClN_2 \cdot 2HCl$: 463.87), calculated on the anhydrous basis.

Description Meclizine Hydrochloride Hydrate is a white to pale yellow crystalline powder, has a slight odor and has no taste.

Meclizine Hydrochloride Hydrate is freely soluble in pyridine, in chloroform or in a mixture of acid, ethanol (95) and water, slightly soluble in dilute acid or in ethanol (95) and practically insoluble in water or in ether.

Identification (1) Determine the absorption spectra of solutions of Meclizine Hydrochloride Hydrate and Meclizine Hydrochloride Hydrate RS in a diluted hydrochloric acid TS (1 in 100) (1 in 100000), as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Meclizine Hydrochloride Hydrate and Meclizine Hydrochloride Hydrate RS as directed in the potassium bromide disk method under Infrared Spectrometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Dissolve 25 mg of Meclizine Hydrochloride Hydrate in a mixture of 2 mol/L nitric acid and ethanol (3 : 5): it responds to the Qualitative Tests for chloride.

Purity ***Related substances***—Weigh accurately a portion of Meclizine Hydrochloride Hydrate, dissolve in the mobile phase to make a solution containing 0.5 mg per mL and use this solution as the test solution. Separately, weigh accurately a portion of Meclizine Hydrochloride Hydrate RS (previously determine the content of water), add mobile phase to make a solution containing 2.5 μg per mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine peak areas of both solutions: total area of the peaks other than the peak of meclizine hydrochloride from the test solution is not more than 2 times of the peak area of meclizine hydrochloride from the standard solution (not more than 1.0 %) and each peak area of the peaks is not larger than the peak area of meclizine hydrochloride from the standard solution (not more than 0.5 %).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Mobile phase: Dissolve 1.5 g of *l*-heptansulfonic acid in 300 mL of water, add 700 mL of acetonitrile and mix. Titrate the solution to pH 4 with 0.05 mol/L of sulfuric acid.

Flow rate: 1.3 mL/minute.

System suitability

System performance: Weigh a volume of Meclizine Hydrochloride Hydrate and 4-chlorobenzo-phenone, dissolve in mobile phase to make the solution containing 10 μg per mL. When the procedure is run with 20 μL of this solution according to the above operating conditions, meclizine hydrochloride and 4-chlorobenzophenone are eluted in this order, with the resolution between the peaks of meclizine hydrochloride and 4-chlorobenzophenone being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution according to the above operating conditions, the relative standard deviation of the peak areas of meclizine hydrochloride is not more than 1.5 %.

Water Not more than 5.0 % (0.5 g, volumetric titration, direct titration).

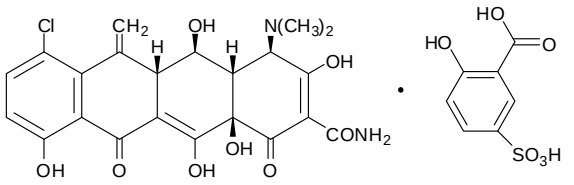
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.35 g of Meclizine Hydrochloride Hydrate, dissolve in 50 mL of chloroform, add 50 mL of acetic acid (100), 5 mL of acetic anhydride and 10 mL of mercuric acetate TS, mix and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 23.195 mg of $C_{25}H_{27}ClN_2 \cdot 2HCl$

Containers and Storage ***Containers***—Tight containers.

Meclocycline Sulfosalicylate



$C_{22}H_{21}ClN_2O_7 \cdot C_7H_6O_6S$: 695.05

(4*R*,4a*S*,5*R*,5a*S*,12a*R*)-7-Chloro-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methylene-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide; 2-hydroxy-5-sulfobenzoic acid [*73816-42-9*]

Meclocycline Sulfosalicylate contains not less than 620 μg (potency) per mg of meclocycline ($C_{22}H_{21}ClN_2O_8$: 476.87).

Description Meclocycline Sulfosalicylate appears as pale yellow powder, is tasteless and odorless.

Meclocycline Sulfosalicylate is sparingly soluble in warm propylene glycol and practically insoluble in water.

Meclocycline Sulfosalicylate dissolves slightly in dilute inorganic acids.

Identification (1) To 5 mg (potency) of Meclocycline Sulfosalicylate, add 2 mL of sulfuric acid: an orange color develops.

(2) To 5 mg (potency) of Meclocycline Sulfosalicylate, add 5 mL of water, mix and add 1 drop of iron (III) chloride TS: a brown color develops.

(3) Determine the absorption spectrum of a solution of Meclocycline Sulfosalicylate in 0.01 mol/L hydrochloric acid-methanol TS (25 in 1000) as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima at 238 nm, 318 nm, 345 nm and 368 nm.

(4) Determine the infrared spectra of Meclocycline Sulfosalicylate and Meclocycline RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Crystallinity Test It meets the requirement.

pH The pH of a suspension obtained by suspending 0.1 g (potency) of Meclocycline Sulfosalicylate in 10 mL of water is between 2.5 and 3.5.

Water Not more than 4.0 % (0.2 g, volumetric titration, direct titration). Use a mixture of carbon tetrachloride, chloroform and methanol (2:2:1) as the solvent instead of methanol.

Assay Weigh accurately about 25 mg (potency) each of Meclocycline Sulfosalicylate and Meclocycline RS and dissolve each in methanol to make exactly 50 mL. Pipet 3.0 mL each of these solutions, add the mobile phase to make exactly 25 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the peak areas, A_T and A_S, of meclocycline in the test solution and the standard solution.

$$\text{Amount [μg (potency)] of meclocycline } (C_{22}H_{21}ClN_2O_8) = \text{Amount [μg (potency)] of Meclocycline RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 340 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography.

Mobile phase: A mixture of 0.001 mol/L ammonium edetate and tetrahydrofuran (85:15)

Flow rate: 0.8 mL/minute

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Meclocycline Sulfosalicylate Cream

Meclocycline Sulfosalicylate Cream contains not less than 90.0 % and not more than 120.0 % of the labeled amount of meclocycline ($C_{22}H_{21}ClN_2O_8$: 476.87).

Method of Preparation Prepare as directed under Creams, with Meclocycline Sulfosalicylate.

Identification Dissolve 10 mg (potency) each of Meclocycline Sulfosalicylate Cream and Meclocycline RS in 100 mL of methanol, filter and use these solutions as the test solution and the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot the test solution and the standard solution on a plate of silica gel for thin-layer chromatography and develop the plate with 0.1 mol/L hydrochloric acid-methanol TS. Examine under ultraviolet light (main wavelength: 254 nm): the spots obtained from the test solution and the standard solution show the same R_f value.

Assay Weigh accurately an amount of Meclocycline Sulfosalicylate Cream, equivalent to about 5 mg (potency) according to the labeled potency, and transfer to a glass-stoppered centrifuge tube. Add 20 mL of methanol and 20 mL of 0.025 mol/L sulfuric acid, extract with a ultrasonicator, shake for 15 minutes with a ultrasonicator and transfer to a 50 mL volumetric flask. Wash the centrifuge tube with two 5 mL volumes of methanol, add the washings to the flask and add methanol to make exactly 50 mL. Transfer a portion of this solution to a suitable centrifuge tube, centrifuge for 5 minutes, pipet 5 mL of this solution and add the mobile phase to make exactly 50 mL. Filter this solution through a membrane filter with a pore size of not more than 0.5 μm and use the filtrate as the test solution. Separately, weigh accurately about 25 mg (potency) of Meclocycline RS and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the peak areas, A_T and A_S, of meclocycline in the test solution and the standard solution.

$$\text{Amount [μg (potency)] of meclocycline per mg of Meclocycline Sulfosalicylate Cream} = \frac{A_T}{A_S} \times \frac{\text{Amount [μg (potency)] of Meclocycline RS taken}}{\text{Amount (mg) of Meclocycline Sulfosalicylate Cream taken}}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 340 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography.

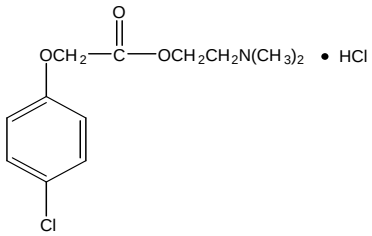
Mobile phase: A mixture of 0.001 mol/L ammonium edetate and tetrahydrofuran (85:15)

Flow rate: 0.8 mL/minute

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Meclofenoxate Hydrochloride



$C_{12}H_{16}ClNO_3 \cdot HCl$: 294.17

2-Dimethylaminoethyl (4-chlorophenoxy)acetate hydrochloride [*3685-84-5*]

Meclofenoxate Hydrochloride contains not less than 98.0 % and not more than 101.0 % of meclofenoxate hydrochloride ($C_{12}H_{16}ClNO_3 \cdot HCl$), calculated on the anhydrous basis.

Description Meclofenoxate Hydrochloride appears as white crystals or crystalline powder. Meclofenoxate Hydrochloride has a pale, characteristic odor and a bitter taste.

Meclofenoxate Hydrochloride is freely soluble in water or in ethanol (95), sparingly soluble in acetic anhydride and practically insoluble in ether.

pH—The pH of a solution of Meclofenoxate Hydrochloride (1 in 20) is between 3.5 and 4.5.

Identification (1) To 10 mg of Meclofenoxate Hydrochloride, add 2 mL of ethanol (95), dissolve by warming, if necessary, cool, add 2 drops of a saturated solution of hydroxylamine hydrochloride in ethanol and 2 drops of a saturated solution of potassium hydroxide in ethanol and heat on a water-bath for 2 minutes. After cooling, render the solution slightly acidic with dilute hydrochloric acid and add 3 drops of iron (III) chloride TS: a red-purple to dark purple color is observed.

(2) Dissolve 50 mg of Meclofenoxate Hydrochloride in 5 mL of water and add 2 drops of Reinecke salt TS: a pale red precipitate is produced.

(3) Determine the absorption spectra of the solutions of Meclofenoxate Hydrochloride and Meclofenoxate Hydrochloride RS (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) A solution of Meclofenoxate Hydrochloride (1 in 100) responds to the Qualitative Tests for chloride.

Melting Point 139 ~ 143 °C.

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Meclofenoxate Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) ***Sulfate***—Perform the test with 1.0 g of Meclofenoxate Hydrochloride. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048 %).

(3) ***Heavy metals***—Proceed with 1.0 g of Meclofenoxate Hydrochloride according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Arsenic***—Prepare the test solution with 1.0 g of Meclofenoxate Hydrochloride according to Method 3 and perform the test (not more than 2 ppm).

(5) ***Organic acids***—Take 2.0 g of Meclofenoxate Hydrochloride, add 50 mL of ether, shake for 10 minutes, filter through a glass filter (G3), wash the residue with two 5 mL volumes of ether and combine the washings with the filtrate. To this solution, add 50 mL of neutralized ethanol and 5 drops of phenolphthalein TS and neutralize with 0.1 mol/L sodium hydroxide VS: the volume of 0.1 mol/L sodium hydroxide VS consumed is not more than 0.54 mL.

Water Not more than 0.5 % (1 g, volumetric titration, direct titration).

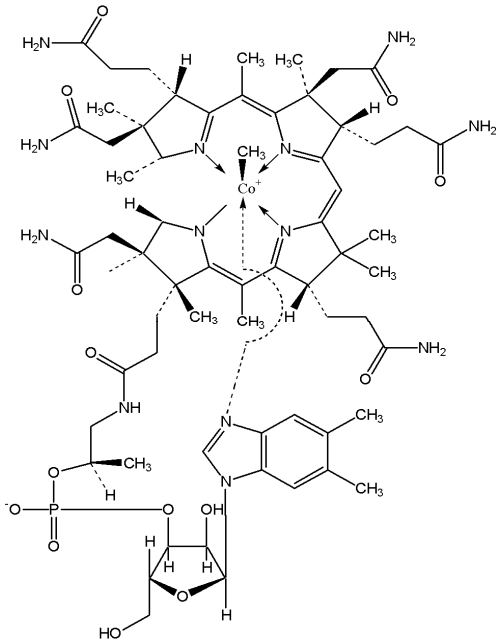
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.4 g of Meclofenoxate Hydrochloride, dissolve in 70 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from blue-green through yellow-green to pale greenish yellow [indicator: 3 drops of a solution of malachite green in acetic acid (100) (1 in 100)]. Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.417 mg of $C_{12}H_{16}ClNO_3 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Mecobalamin



$C_{63}H_{91}CoN_{13}O_{14}P$: 1344.38

Carbanide;Cobalt(3+);[5-(5,6-Dimethylbenz-imidazol-1-yl)-4-hydroxy-2-(hydroxymethyl)oxolan-3-yl]-1-[3-[(4*Z*,9*Z*,14*Z*)-2,13,18-*tris*(2-amino-2-oxoethyl)-7,12,17-*tris*(3-amino-3-oxopropyl)-3,5,8,8,13,15,18,19-octamethyl-2,7,12,17-tetrahydro-1*H*-corrin-21-id-3-yl]propanoylamino]propan-2-ylphosphate [*13422-55-4*]

Mecobalamin contains not less than 98.0 % and not more than 101.0 % of mecobalamin ($C_{63}H_{91}CoN_{13}O_{14}P$).

Description Mecobalamin appears as dark red crystals or crystalline powder.
Mecobalamin is sparingly soluble in water, slightly soluble in ethanol (99.5), and practically insoluble in acetonitrile.
Mecobalamin is affected by light.

Identification (1) Perform this procedure without exposure to light, using light-resistant vessels. Determine the absorption spectra of the solutions of Mecobalamin and Mecobalamin RS in hydrochloric acid-potassium chloride buffer solution, pH 2.0 (1 in 20000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectra of the solutions of Mecobalamin and Mecobalamin RS in phosphate buffer solution, pH 7.0 (1 in 20000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Mix 1 mg of Mecobalamin with 50 mg of potassium bisulfate, and fuse by igniting. Cool, break up the mass with a glass rod, add 3 mL of water, and dissolve by boiling. Add 1 drop of phenolphthalein TS, then add dropwise sodium hydroxide TS until a pale red color just develops. Add 0.5 g of sodium acetate trihydrate, 0.5 mL of dilute acetic acid and 0.5 mL of a solution of disodium 1-nitroso-2-naphthol-3,6-disulfonate (1 in 500): a red to orange color is immediately produced. Then add 0.5 mL of hydrochloric acid, and boil for 1 minute: the red color does not disappear.

Purity (1) ***Clarity and color of solution***—Dissolve 20 mg of Mecobalamin in 10 mL of water: the solution is clear and red in color.

(2) ***Related substances***—Perform the test with 10 μL of the test solution obtained in the Assay as directed under Liquid Chromatography according to the following conditions. Determine the peak area of mecobalamin and others of the test solution by the automatic integration method: each area of the peaks other than mecobalamin is not larger than 0.5 % of the peak area of mecobalamin, and the total area of the peaks other than mecobalamin is not larger than 2.0 %.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability

Test for required detectability: To exactly 1 mL of the test solution, add the mobile phase to make exactly 100 mL, and use this solution as the test solution for system suitability. Pipet 1 mL of the test solution for system suitability, add the mobile phase to make exactly 100 mL. Confirm that the peak area of mecobalamin from 10 μL of this solution is equivalent to 7 to 13 % of that of mecobalamin from 10 μL of the test solution for system suitability.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the testis repeated 6 times with 10 μL each of the test solution for system suitability under the above operating conditions, the relative standard deviation of the peak areas of mecobalamin is not more than 3.0 %.

Time span of measurement: About 2.5 times as long as the retention time of mecobalamin.

Water Not more than 12 % (0.1 g, volumetric titration, direct titration).

Assay Perform this procedure without exposure to light, using light-resistant vessels. Weigh accurately about 50 mg of Mecobalamin and Mecobalamin RS (separately, determine the water in the same manner as Mecobalmin), dissolve each in the mobile phase to make exactly 50 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with exactly 10 μL of each of the test solution and the standard solution as directed under Liq-

uid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of mecobalamin in each solution.

$$\text{Amount (mg) of } C_{63}H_{91}CoN_{13}O_{14}P = \text{Amount (mg) of Mecobalamin RS, calculated on the anhydrous basis} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 266 nm)

Column: A stainless steel column 4.6 mm in internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: To 200 mL of acetonitrile, add 800 mL of 0.02 mol/L phosphate buffer solution, pH 3.5, then add 3.76 g of sodium 1-hexane sulfonate to dissolve.

Flow rate: Adjust the flow rate so that the retention time of mecobalamin is about 12 minutes.

System suitability

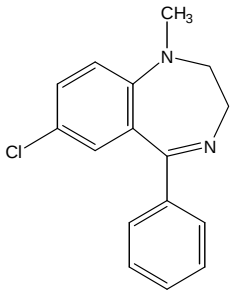
System performance: Dissolve 5 mg each of cyanocobalamin and hydroxocobalamin acetate in the mobile phase to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, cyanocobalamin and hydroxocobalamin are eluted in this order with the resolution between these peaks being not less than 3. In addition, when the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of mecobalamin is not less than 6000.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of mecobalamin is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Medazepam



$C_{16}H_{15}ClN_2$: 270.76

7-Chloro-1-methyl-5-phenyl-2,3-dihydro-1*H*-benzo[1,4]diazepine [*2898-12-6*]

Medazepam, when dried, contains not less than 98.5 % and not more than 101.0 % of medazepam ($C_{16}H_{15}ClN_2$).

Description Medazepam appears as white to pale yellow crystals or crystalline powder and is odorless.
Medazepam is freely soluble in methanol, in ethanol, in acetic acid (100) or in ether and practically insoluble in water.
Medazepam is gradually colored by light.

Identification (1) Dissolve 10 mg of Medazepam in 3 mL of citric acid-acetic acid TS: a deep orange color is observed. Heat on a water-bath for 3 minutes: the color changes to dark red.

(2) Determine the absorption spectra of the solutions of Medazepam and Medazepam RS in methanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Medazepam and Medazepam RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(4) Perform the test with Medazepam as directed under the Flame Coloration Test (2): a green color is observed.

Melting Point 101 ~ 104 °C

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Medazepam in 10 mL of methanol: the solution is clear and pale yellow to yellow in color.

(2) ***Chloride***—Dissolve 1.5 g of Medazepam in 50 mL of ether, add 46 mL of water and 4 mL of sodium carbonate TS, shake and collect the water layer. Wash the water layer with two 20-mL volumes of ether and filter. To 20 mL of the filtrate, add diluted nitric acid to neutralize, add 6 mL of diluted nitric acid and water to make 50 mL and perform the test. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018 %).

(3) ***Heavy metals***—Proceed with 1.0 g of Medazepam according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Arsenic***—Prepare the test solution with 1.0 g of Medazepam according to Method 3 and perform the test (not more than 2 ppm).

(5) ***Related substances***—Dissolve 0.25 g of Medazepam in 10 mL of methanol and use this solution as the test solution. Pipet 1 mL of the test solution and add methanol to make exactly 20 mL. Pipet 2 mL

of this solution, add methanol to make exactly 50 mL and use this solution as the standard solution. Perform the test with the test solution and standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, acetone and strong ammonia water (60 : 40 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, 60 °C, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.4 g of Medazepam, previously dried, dissolve in 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Methods in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 27.076 mg of $C_{16}H_{15}ClN_2$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Medicinal Carbon

Description Medicinal Carbon is a black, odorless and tasteless powder.

Identification Place 0.5 g of Medicinal Carbon in a test tube and heat by direct application of flame with the aid of a current of air: it burns without any flame. Pass the evolved gas through calcium hydroxide TS: a white turbidity is produced.

Purity (1) ***Acid or alkali***—Boil 3.0 g of Medicinal Carbon with 60 mL of water for 5 minutes, allow to cool, dilute to 60 mL with water and filter: the filtrate is colorless and neutral.

(2) ***Chloride***—Take 4.0 mL of the filtrate obtained in (1) in a Nessler tube, add 6 mL of dilute nitric acid and sufficient water to make 50 mL and perform the test. Prepare the control solution with 0.80 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.142 %).

(3) ***Sulfate***—Take 5 mL of the filtrate obtained in (1) in a Nessler tube, add 1 mL of dilute hydrochloric acid and sufficient water to make 50 mL and perform the test. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric aicd VS (not more than 0.192 %).

(4) ***Sulfide***—Boil 0.5 g of Medicinal Carbon with a mixture of 15 mL of dilute hydrochloric acid and 10 mL of water: lead acetate paper does not become brown when held in the evolved gas within 5 minutes.

(5) ***Cyanogen compounds***—Place a mixture of 5 g of Medicinal Carbon, 2 g of L-tartaric acid and 50 mL of water in a distilling flask connected to a condenser provided with a tightly fitting adapter, the end of which dips below the surface of a mixture of 2 mL of sodium hydroxide TS and 10 mL of water, contained in a small flask surrounded by ice. Heat the mixture in the distilling flask to boiling and distil to 25 mL. Dilute the distillate with water to make 50 mL. To 25 mL of the diluted distillate, add 1 mL of iron (II) sulfate heptahydrate solution (1 in 20), heat the mixture almost to boiling, cool and filter. To the filtrate, add 1 mL of hydrochloric acid and 0.5 mL of dilute iron (III) chloride TS: no blue color is produced.

(6) ***Acid soluble substances***—Take about 1 g of Medicinal Carbon, accurately weighed, add 20 mL of water and 5 mL of hydrochloric acid, boil for 5 minutes, filter, wash the residue with 10 mL of hot water and add the washings to the filtrate. Add 5 drops of sulfuric acid to the filtrate, evaporate to dryness and ignite the residue strongly: the residue is not more than 3.0 %.

(7) ***Heavy metals***—Proceed with 0.5 g of Medicinal Carbon according to Method 2 and perform the test. Prepare the control solution with 2.5 mL of standard lead solution (not more than 50 ppm).

(8) ***Zinc***—Ignite 0.5 g of Medicinal Carbon to ash, add 5 mL of dilute nitric acid to the residue, boil gently for 5 minutes, filter, wash with 10 mL of water and combine the washings and the filtrate. Add 3 mL of ammonia TS to the solution, filter again, wash with water, combine the washings and the filtrate, add another washing to make 25 mL, add 1 drop of sodium sulfide TS and allow to stand for 3 minutes: the liquid produces no turbidity.

(9) ***Arsenic***—Prepare the test solution with 1.0 g of Medicinal Carbon according to Method 3 and perform the test (not more than 2 ppm).

Loss on Drying Not mort than 15.0 % (1 g, 105 °C, 4 hours).

Residue on Ignition Not more than 4.0 % (1 g).

Adsorptive Power (1) Add 1.0 g of Medicinal Carbon, previously dried, to 100 mL of water containing 120 mg of Quinine Sulfate, shake the mixture vigorously for 5 minutes, filter immediately and discard the first 20 mL of the filtrate. Add 5 drops of iodine TS to 10 mL of the subsequent filtrate: no turbidity is produced.

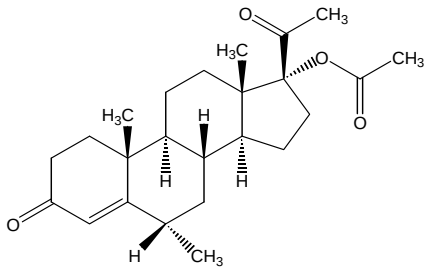
(2) Dissolve 0.25 g of methylene blue, exactly weighed, in water to make exactly 250 mL. Measure two 50 mL volumes of this solution into each of two glass-stoppered flasks, To one flask, add exactly 0.25 g of Medicinal Carbon, previously dried and weighed accurately, and shake vigorously for 5 minutes. Filter the contents of each flask, discard the first 20 mL of

each filtrate. Pipet 25 mL of the subsequent filtrate into two 250 mL volumetric flasks. To each volumetric flask, add 50 mL of a solution of sodium acetate (1 in 10), then add exactly 35 mL of 0.05 mol/L iodine VS with swirling. Allow them to stand for 50 minutes, shaking vigorously from time to time. Dilute each mixture to exactly 250 mL with water, allow to stand for 10 minutes and filter each solution at a temperature not exceeding 20 °C, discard the first 30 mL of each filtrate. Titrate the excess iodine in 100 mL of each filtrate with 0.1 mol/L sodium thiosulfate VS. The difference between the two titrations is not less than 1.2 mL.

Microbial Limit The total aerobic microbial count is not more than 1000 CFU/g, the total combined yeasts/mould count is not more than 100 CFU/g.

Containers and Storage ***Containers***—Well-closed containers.

Medroxyprogesterone Acetate



$C_{24}H_{34}O_4$: 386.52

[(6*S*,8*R*,9*S*,10*R*,13*S*,14*S*,17*R*)-17-Acetyl-6,10,13-trimethyl-3-oxo-2,6,7,8,9,11,12,14,15,16-decahydro-1*H*-cyclopenta[a]phenanthren-17-yl]acetate [*71-58-9*]

Medroxyprogesterone Acetate, contains not less than 97.0 % and not more than 103.0 % of medroxyprogesterone acetate ($C_{24}H_{34}O_4$), calculated on the dried basis.

Description Medroxyprogesterone Acetate is a white crystalline powder and is odorless.

Medroxyprogesterone Acetate is freely soluble in chloroform and soluble in acetone or in 1,4-dioxane and sparingly soluble in ethanol (95) or in methanol and slightly soluble in ether and practically insoluble in water.

Medroxyprogesterone Acetate is stable in air.

Melting Point—About 205 °C

Identification (1) Determine the absorption spectra of solutions of Medroxyprogesterone Acetate and Medroxyprogesterone Acetate RS in ethanol (95) (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths. And the difference in absorption at 241 nm, calculated on the dried basis, is not more than 2.0 %.

(2) Determine the infrared spectra of Medroxyprogesterone Acetate and Medroxyprogesterone Acetate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +47 ~ +53° (0.250 g after drying, acetone, 25 mL, 100 mm).

Purity (1) ***Related substance I***—Weigh accurately about 0.20 g of Medroxyprogesterone Acetate, dissolve in dichloromethane to make exactly 10 mL and use this solution as the test solution. Separately, weigh accurately 0.20 g of Medroxyprogesterone Acetate RS and 1.0 mg of medroxyprogesterone acetate related substance I RS, dissolve in dichloromethane to make 10 mL and use this solution as the standard solution. Proceed with these solutions as directed under the Thin-layer Chromatography. Apply 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate using a mixture of hexane, *tert*-butylmethyl ether and tetrahydrofuran (45 : 45 : 10) to a distance of about 10 cm and air-dry the plate. Heat the plate to dry at 120 °C for 10 minutes, spray evenly the plate with a solution of *p*-toluenesulfonic acid monohydrate in ethanol (95) (1 in 5), heat the plate at 120 °C for 10 minutes and examine the plate under ultraviolet light (main wavelength: 365 nm). A large blue fluorescent spot with an R_f value higher than that of the principal spot from the test solution is not more intense that the corresponding blue fluorescent spot obtained from the standard solution (not more than 0.5 %).

(2) ***Related substances II***—Dissolve about 62.5 mg of Medroxyprogesterone Acetate, accurately weighed, in 25 mL of the mobile phase and use this solution as the test solution. Separately, weigh accurately a portion of Medroxyprogesterone Acetate RS and dissolve in mobile phase so that each mL contains 50 μg of Medroxyprogesterone Acetate RS and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine peak area of each peak from the test solution and the standard solution: the area of each peak other than the principal peak is from the test solution not more than 1 % and the total area of the peaks other than the principal peak from the test solution is not more than 1.5 %.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (about 3μm to 10 μm in particle diameter)

Mobile phase: A mixture of acetonitrile and water (3 : 2).

Flow rate: 1 mL/minute.

System suitability

System performance: Weigh a suitable portion of Megesterol Acetate RS and Medroxyprogesterone Acetate RS and dissolve in the mobile phase to render the concentration of 40 μg in 1 mL each. When the procedure is run with 20 μL of this solution according to the above operating conditions, the resolution between megesterol acetate and medroxyprogesterone acetate is not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution according to the above operating conditions, the relative standard deviation of the peak areas of medroxyprogesterone acetate is not more than 3.0 %.

Loss on Drying Not more than 1.0 % (1 g, 105 °C, 3 hours)

Assay Weigh accurately about 25 mg of Medroxyprogesterone Acetate, dissolve in acetonitrile to make exactly 25 mL and use this solution as the test solution. Separately, weigh accurately about 25 mg of Medroxyprogesterone Acetate RS, previously dried at 105 °C for 3 hours, dissolve in acetonitrile to make exactly 25 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine the peak areas, A_T and A_S, of medroxyprogesterone acetate for the test solution and the standard solution, respectively.

Amount (mg) of medroxyprogesterone acetate

$$(C_{24}H_{34}O_4) = 25 \times C \times \frac{A_T}{A_S}$$

C: Concentration(mg/mL) of the standard solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm to 10 μm in particle diameter)

Mobile phase: A mixture of water and acetonitrile (60 : 40).

Flow rate: 2 mL/minute.

System suitability

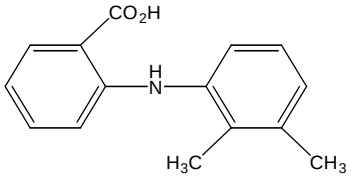
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the symmetry factor for the peak of medroxyprogesterone acetate is not more than 2.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Mefenamic Acid



$C_{15}H_{15}NO_2$: 241.29

2-((2,3-Dimethylphenyl)amino)benzoic acid [*61-68-7*]

Mefenamic Acid, when dried, contains not less than 99.0 % and not more than 101.0 % of mefenamic acid ($C_{15}H_{15}NO_2$).

Description Mefenamic Acid is a white to pale yellow powder, is odorless and tasteless at first, but leaves a slightly bitter aftertaste.

Mefenamic Acid is sparingly soluble in ether, slightly soluble in methanol, in ethanol (95) or in chloroform and practically insoluble in water.

Mefenamic Acid dissolves in sodium hydroxide TS.

Melting point—About 225 °C (with decomposition).

Identification (1) Dissolve 10 mg of Mefenamic Acid in 1 mL of methanol by warming, cool, add 1 mL of a solution of 4-nitrobenzenediazonium fluoroborate (1 in 1000) and 1 mL of sodium hydroxide TS and mix thoroughly: an orange-red color is observed.

(2) Dissolve 10 mg of Mefenamic Acid in 2 mL of sulfuric acid and heat: a yellow color and green fluorescence is observed.

(3) Dissolve 7 mg each of Mefenamic Acid and Mefenamic Acid RS in a solution of hydrochloric acid in methanol (1 in 1000) to make 500 mL. Determine the absorption spectra of both solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) ***Chloride***—Take 1.0 g of Mefenamic Acid, add 20 mL of sodium hydroxide TS and dissolve by warming. Cool, add 2 mL of acetic acid (100) and water to make exactly 100 mL, mix well and filter. Discard the first 10 mL of the filtrate, take subsequent 25 mL of the filtrate and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the standard solution. Prepare the control solution as follows: take 0.50 mL of 0.01 mol/L hydrochloric acid VS, add 5 mL of sodium hydroxide TS, 0.5 mL of acetic acid (100), 6 mL of dilute nitric acid and wa-

ter to make 50 mL (not more than 0.071 %).

(2) ***Heavy metals***—Proceed with 2.0 g of Mefenamic Acid according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Mefenamic Acid according to Method 3 and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 0.10 g of Mefenamic Acid in 5 mL of a mixture of chloroform and methanol (3 : 1) and use this solution as the test solution. Pipet 1.0 mL of the test solution, add a mixture of chloroform and methanol (3 : 1) to make exactly 200 mL. Pipet 10.0 mL of this solution, add a mixture of chloroform and methanol (3 : 1) to make exactly 50 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 25 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 2-methyl-1-propanol and ammonia solution (28) (3 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, P_2O_5, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.5 g of Mefenamic Acid, previously dried, dissolve in 100 mL of neutralized ethanol by warming gently. Cool and titrate with 0.1 mol/L sodium hydroxide VS until the color of the solution changes from yellow through yellow-red to red-purple (indicator: 2 to 3 drops of phenol red TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 24.129 mg of $C_{15}H_{15}NO_2$

Containers and Storage ***Containers***—Well-closed containers.

Mefenamic Acid Capsules

Mefenamic Acid Capsules contain not less than 90.0 % and not more than 110.0 % of the labeled amount of mefenamic acid ($C_{15}H_{15}NO_2$: 241.29).

Method of Preparation Prepare as directed under Capsules, with Mefenamic Acid.

Identification (1) Take out contents of Mefenamic Acid Capsules and mix well, weigh a portion of the contents equivalent to 0.25 g of Mefenamic Acid according to the labeled amount, dissolve in 100 mL of a mixture of chloroform and methanol (3 : 1), shaking vigorously and add a mixture of chloroform and methanol (3 : 1) to make 250 mL and filter. Use this filtrate as the test solution. Separately, weigh 25 mg of Mefenamic Acid RS, dissolve in a mixture of chloroform and methanol (3 : 1) to make 25 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatograhy. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, ethyl acetate and acetic acid (100) (75 : 25 : 1) to a distance of about 10 cm and air-dry the plate. Expose the plate to fume of iodine vapor: the R_f values and the intensities of the spots obtained from the test solution correspond to those from the standard solution.

(2) Proceed as directed in the Assay, both spectra of the test solution and the standard solution exhibit similar peaks at the same retention time.

Dissolution Test Perform the test with 1 capsule of Mefenamic Acid Capsules at 100 revolutions per minute according to Method 1 under the Dissolution Test, using 900 mL of 0.05 mol/L tris buffer as the dissolution solution. Take the dissolved solution after 45 minutes from the start of the test and filter. Pipet a suitable portion of the filtrate, dilute with the dissolution solution to make the concentration of about 0.2 mg per mL and use this solution as the test solution. Separately, weigh a suitable amount of Mefenamic Acid RS, dissolve in the dissolution solution to give the same concentration as the test solution and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Assay.
The dissolution rate of Mefenamic Acid Capsules in 45 minutes is not less than 75 %.

0.05 mol/L tris buffer—Weigh 60.5 g of 2-amino-2-hydroxymethyl-1,3-propane-diol dissolve in 6 L of water and add water to make 10 L. Adjust the pH with phosphoric acid to 9.0 ± 0.05. Dissolve 100 g of soium lauryl sulfate in 6 L of the solution and mix this solution with the first solution.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately the contents of not less than 20 Mefenamic Acid Capsules. Weigh accurately a portion of the contents equivalent to about 50 mg of mefenamic acid ($C_{15}H_{15}NO_2$), add 5 mL of tetrahydrofuran and dissolve using an ultrasonic wave for 5 minutes, add mobile phase to make exactly 250 mL, filter and use the filtrate as the test solution. Separately, weigh accurately about 50 mg of Mefenamic Acid RS,

dissolve in 5 mL of tetrahydrofuran by shaking, add the mobile phase to make exactly 250 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and measure the peak areas, A_T and A_S, of Mefenamic Acid for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of mefenamic acid } (C_{15}H_{15}NO_2) = \text{Amount (mg) of Mefenamic Acid RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm to 10 μm in particle diameter).

Mobile phase: A mixture of acetonitrile, buffer solution and tetrahydrofuran (23: 20: 7)

Flow rate: 1 mL/minute.

System suitability

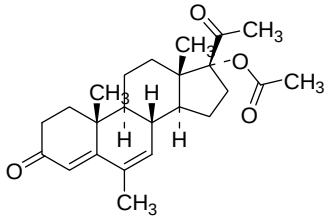
System performance: When the procedure is run with 10 μL of the standard solution according to the above operating conditions, the symmetry factor for the peak of mefenamic acid is not more than 1.6.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution according to the above operating conditions, the relative standard deviation of the peak areas of mefenamic acid is not more than 1.0 %.

Buffer solution—Dissolve 5.7 g of monobasic ammonium phosphate in 1000 mL of water. Adjust the pH with ammonia TS to 5.0.

Containers and Storage ***Containers***—Well-closed containers.

Megestrol Acetate



$C_{24}H_{32}O_4$: 384.51

[(8*R*,9*S*,10*R*,13*S*,14*S*,17*R*)-17-Acetyl-6,10,13-trimethyl-3-oxo-2,8,9,11,12,14,15,16-octa-hydro-1*H*-cyclopenta[a]phenanthren-17-yl]acetate [*595-33-5*]

Megestrol Acetate contains not less than 97.0 % and not more than 103.0 % of megestrol acetate ($C_{24}H_{32}O_4$), calculated on the anhydrous basis.

Description Megestrol Acetate appears as white or almost white crystalline powder.
Megestrol Acetate is soluble in acetone, sparingly soluble in ethanol (95) and practically insoluble in water.

Identification Determine the infrared spectra of Megestrol Acetate and Megestrol Acetate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +8.8 ~ +12.0° (200 mg after drying, chloroform, 10 mL, 100 mm).

Melting Point 213 ~ 220 °C

Purity ***Heavy metals***—Proceed with 1.0 g of Megestrol Acetate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

Water Not more than 0.5 % (0.25 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.2 % (1 g, 600 °C)

Assay Weigh accurately about 0.1 g of Megestrol Acetate and add acetonitrile to make 100 mL. Pipet 4 mL of this solution, add 5 mL of the internal standard solution, add the mobile phase to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately about 0.1 g of Megestrol Acetate RS and add acetonitrile to make 100 mL. Pipet 4 mL of this solution, add 5 mL of the internal standard solution, add the mobile phase to make exactly 50 mL and use this solution as the standard solution. Perform the test with 25 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the ratios, Q_T and Q_S, of the peak area of megestrol acetate to that of the internal standard.

$$\text{Amount (mg) of megestrol acetate } (C_{24}H_{32}O_4) = \text{Amount (mg) of megestrol acetate RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of propylparaben in acetonitrile (8 in 10000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm)

Column: A stainless steel column 3.9 mm in internal diameter and 30 cm in length packed with

octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of acetonitrile and water (55:45)

Flow rate: 1 mL/minute

System suitability

System performance: When the procedure is run with 25 μL of the standard solution under the above operating conditions, the relative retention time of propylparaben with respect to megestrol acetate is about 0.4 with the resolution between their peaks being not less than 8.0.

System repeatability: When the test is repeated 6 times with 25 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Megestrol Acetate Oral Suspension

Megestrol Acetate Oral Suspension contains not less than 90.0 % and not more than 110.0 % of the labeled amount of megestrol acetate ($C_{24}H_{32}O_4$: 384.51).

Method of Preparation Prepare as directed under Suspensions, with Megestrol Acetate.

Identification Take a volume of Megestrol Acetate Oral Suspension, equivalent to 160 mg of megestrol acetate according to the labeled amount, transfer to a separatory funnel, add 50 mL of water and 40 mL of chloroform, shake, allow to separate and use the chloroform layer as the test solution. Separately, weigh accurately 40 mg of Megestrol Acetate RS, dissolve in chloroform to make 10 mL and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethyl acetate (4 : 1) and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spots obtained from the test solution and the standard solution show the same R_f value.

pH 3.0 ~ 4.7.

Dissolution Test Perform the test with V mL of Megestrol Acetate Oral Suspension, equivalent to 160 mg of megestrol acetate according to the labeled amount, at 25 revolutions per minute according to Method 2, using 900 mL of 5 % sodium lauryl sulfate as the dissolution solution. Take the dissolved solution after 30 minutes from the start of the test, filter through a membrane filter with pore size of not more than 0.45 μm and use the filtrate as the test solution. Separately, weigh accurately about 45 mg of Megestrol Acetate RS, add 12 mL of methanol, allow to stand in a water-bath to dissolve, add the dissolution solution to make exactly 250 mL and use this solution as the standard solution. Dilute the test solution and the standard solution to a suitable concentration with the dissolution solution, if necessary. Determine the absorbances, A_T and A_S, at 292 nm of the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometery, using the dissolution solution as the blank. The dissolution rate of Megestrol Acetate Oral Suspension in 30 minutes is not less than 80 % (Q).

Dissolution rate (%) with respect to the labeled amount of megestrol acetate ($C_{24}H_{32}O_4$)

$$= C \times \frac{A_T}{A_S} \times \frac{V_D}{V} \frac{100}{L}$$

C: Concentration (mg/mL) of the standard solution

V: Volume (mL) of Megestrol Acetate Oral Suspension taken

V_D: Volume of solvent, 900 mL

L: Labeled amount (mg/mL) of Megestrol Acetate Oral Suspension

Microbial Limit It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Take a volume of Megestrol Acetate Oral Suspension, equivalent to 160 mg of megestrol acetate according to the labeled amount, add the mobile phase to make exactly 1000 mL. Pipet 5 mL of this solution, make exactly 10 mL and use this solution as the test solution. Separately, weigh accurately 80 mg of Megestrol Acetate RS, dissolve in the mobile phase to make 1000 mL and use this solution as the standard solution. Perform the test with 25 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the peak areas, A_T and A_S, of megestrol acetate in each solution.

Amount (mg) of megestrol acetate ($C_{24}H_{32}O_4$)

$$= \text{Amount (mg) of Megestrol Acetate RS} \times \frac{A_T}{A_S} \times 2$$

Operating conditions

Detector: Ultraviolet absorption photometer (wavelength: 280 nm)

Column: A stainless steel column 3.9 mm in internal diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of acetonitrile and water (11:9)

Flow rate: 1.5 mL/minute

System suitability

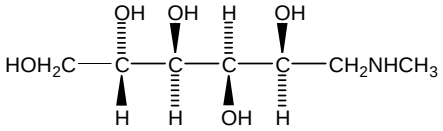
System performance: When the procedure is run with 25 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of megestrol acetate is not less than 2500.

System repeatability: When the test is repeated 6 times with 25 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of megestrol acetate is not more than 2.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Meglumine



$C_7H_{17}NO_5$: 195.21

(2*R*,3*R*,4*R*,5*S*)-6-(Methylamino)hexane-1,2,3,4,5-pentol [*6284-40-8*]

Meglumine, when dried, contains not less than 99.0 % and not more than 101.0 % of meglumine ($C_7H_{17}NO_5$).

Description Meglumine is a white, crystalline powder, is odorless and has a slightly bitter taste.
Meglumine is freely soluble in water and slightly soluble in ethanol (95) and practically insoluble in ether.

pH—The pH of a solution of Meglumine (1 to 10) is between 11.0 and 12.0.

Identification (1) Take 1 mL of a solution of Meglumine (1 in 10) and add 1 mL of potassium 1,2-naphthoquinone-4-sulfonate TS: a deep red color is observed.

(2) Take 2 mL of a solution of Meglumine (1 in 10), add 1 drop of methyl red TS and add 0.5 mL of dilute sodium hydroxide TS and 0.5 g of boric acid after neutralizing with 0.5 mol/L sulfuric acid TS: a deep red color is observed.

(3) Dissolve 0.5 g of Meglumine in 1 mL of diluted hydrochloric acid (1 in 3) and add 10 mL of ethanol (99.5): a white precipitate is produced. Then, rubbing the inside wall with a glass rod, cool with ice and produce more precipitate. Filter the precipitate by suction through a glass filter (G3), wash the precipitate with a small volume of ethanol (99.5) and dry at 105 °C for 1 hour: the residue thus obtained melts between 149 °C and 152 °C.

Specific Optical Rotation $[\alpha]_D^{20}$: -16.0 ~ -17.0° (after drying, 1 g, water, 10 mL, 100 mm).

Melting Point 128 ~ 131 °C.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Meglumine in 10 mL of water: the solution is clear and colorless.

(2) ***Chloride***—Dissolve 1.0 g of Meglumine in 30 mL of water and add 10 mL of dilute nitric acid and water to make 50 mL. Perform the test. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.009 %).

(3) ***Sulfate***—Dissolve 1.0 g of Meglumine in 30 mL of water and add 5 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.019 %).

(4) ***Heavy metals***—Proceed with 2.0 g of Meglumine according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(5) ***Arsenic***—Prepare the test solution with 2.0 g of Meglumine according to Method 3 and perform the test (not more than 1 ppm).

(6) ***Reducing substances***—Take 5 mL of a solution of Meglumine (1 in 20), add 5 mL of Fehling's TS and boil for 2 minutes: no red-brown precipitate is produced.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 4 hours).

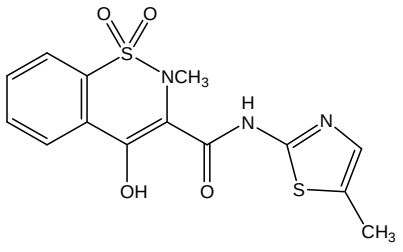
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.4 g of Meglumine, previously dried, dissolve in 25 mL of water and titrate with 0.1 mol/L hydrochloric acid VS (indicator: 2 drops of methyl red TS).

Each mL of 0.1 mol/L hydrochloric acid VS
= 19.521 mg of $C_7H_{17}NO_5$

Containers and Storage ***Containers***—Tight containers.

Meloxicam



$C_{14}H_{13}N_3O_4S_2$: 351.40

4-Hydroxy-2-methyl-*N*-(5-methyl-1,3-thiazol-2-yl)-1,1-dioxo-1λ6,2-benzothiazine-3-carboxamide
[*71125-38-7*]

Meloxicam contains not less than 99.0 % and not more than 100.5 % of meloxicam ($C_{14}H_{13}N_3O_4S_2$), calculated on a dried basis.

Description Meloxicam is a pale yellow powder. Meloxicam is soluble in *N,N*-dimethylformamide, slightly soluble in acetone, very slightly soluble in ethanol (95) or in methanol, and practically insoluble in water.

Identification **(1)** Determine the absorption spectra of the solutions of Meloxicam and Meloxicam RS in methanol (1.5 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Meloxicam and Meloxicam RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity (1) ***Clarity of solution***—The solution obtained by dissolving 0.5 g of Meloxicam in 10 mL of *N,N*-dimethylformamide is clear.

(2) ***Heavy metals***—Proceed with 2.0 g of Meloxicam according to Method 2and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Dissolve 80 mg of Meloxicam in 5 mL of a mixture of methanol and 1 mol/L sodium hydroxide TS (50:3), add methanol to make exactly 20 mL and use this solution as the test solution. Dissolve 12 mg of Meloxicam RS in 5 mL of a mixture of methanol and 1 mol/L sodium hydroxide TS (50:3) and add methanol to make exactly 20 mL. To 2 mL of this solution, add methanol to make exactly 100 mL and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine each peak area in each solution by the automatic integration method and calculate the amount of each related substance in the test solution. In the chromatogram obtained at 350 nm, meloxicam related substance I {ethyl 4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide} is not more than 0.1 %, and each of meloxicam related substance III {*N*-(3,4-dimethylthiazol-2(3*H*)-ylidene)-4-hydroxy-2-methyl-2*H*-benzo[*e*][1,2]thiazine-3-carboxamide 1,1-dioxide} and meloxicam related substance IV {*N*-(3-ethyl-5-methylthiazol-2(3*H*)-ylidene)-4-hydroxy-2-methyl-2*H*-benzo[*e*][1,2]thiazine-3-carboximide 1,1-dioxide} is not more than 0.05 %. Use the peak area of meloxicam related substance I after dividing the area calculated by the automatic integration method by its response factor, 0.5. In the chromatogram obtained at 260 nm, meloxicam related substance II {5-methylthiazol-2-ylamine} from the test solution is not more than 0.1 %. Any related substance other than meloxicam from the test solution obtained at 350 nm and at 260 nm and other than the peaks mentioned above is not more than 0.1 % and the total of all related substances is not more than 0.3 %.

$$\text{Amount (\%) of each related substance} = 100 \times \frac{A_i}{A_S} \times \frac{C_S}{C_T}$$

A_i: Peak area of each related substance in the test solution

A_S: Peak area of meloxicam at 350 nm obtained from the standard solution

C_S: Concentration (mg/mL) of Meloxicam RS in the standard solution

C_T: Concentration (mg/mL) of meloxicam in the test solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 260 nm and 350 nm)

Column: A stainless steel column about 4.6 mm internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45 °C

Mobile phase: Use variable mixtures of mobile A and mobile phase B, and program the chromatograph as follows.

Mobile phase A: Dissolve 1 g of potassium dihydrogen orthophosphate in 1000 mL of water, and adjust the solution pH to 6.0 with 1 mol/L sodium hydroxide TS.

Mobile phase B: Methanol.

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-2	60	40
2-10	60→30	40→70
10-15	30	70
15-15.1	30→60	70→40
15.1-18	60	40

Flow rate: 1.0 mL/minute

System suitability

System performance: Dissolve 4 mg each of Meloxicam RS, Meloxicam Related Substance I RS and Meloxicam Related Substance II RS in 5 mL of a mixture of methanol and 1 mol/L sodium hydroxide TS (50:3), add methanol to make 50 mL and use this solution as the system suitability solution. When the procedure is run with 5 μL of this solution under the above operating conditions, the resolution between the peaks

of meloxicam and related substance I at 350 nm is not less than 3.0, and the resolution between the peak s of meloxicam and related substance II at 260 nm is not less than 3.0. The relative retention time of related substances I, II, III and IV with respect to meloxicam is 1.4, 0.4, 1.7 and 1.9, respectively.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of meloxicam is not more than 10 %.

Loss on Drying Not more than 0.5 % (3 g, 105 °C, constant mass)

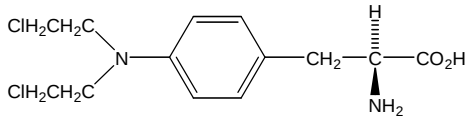
Residue on Ignition Not more than 0.1 % (1 g).

Assay Dissolve 0.25 g of Meloxicam, accurately weighed, in a mixture of 50 mL of acetic acid (100) and 5 mL of anhydrous formic acid, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry).

Each mL of 0.1 mol/L perchloric acid VS
= 35.14 mg of $C_{14}H_{13}N_3O_4S_2$.

Containers and Storage ***Containers***—Well-closed containers.

Melphalan



$C_{13}H_{18}Cl_{12}N_2O_2$: 305.20

(2*S*)-2-Amino-3-[4-[bis(2-chloroethyl)amino]phenyl] propanoic acid [*148-82-3*]

Melphalan contains not less than 93.0 % and not more than 101.0 % of melphalan ($C_{13}H_{18}C_{12}N_2O_2$), calculated on the dried basis.

Description Melphalan is a white to pale yellowish white, crystalline powder.
Mephalan is slightly soluble in water, in methanol or in ethanol (95) and practically insoluble in ether.
Mephalan dissolves in dilute hydrochloric acid or in dilute sodium hydroxide TS.
Mephalan is gradually colored by light.

Specific Optical Rotation— $[\alpha]_D^{20}$: About -32° (0.50 g, calculated on the dried basis, methanol, 100 mL, 100 mm).

Identification (1) Take 20 mg of Melphalan, add 50 mL of methanol, dissolve by warming, add 1 mL of a solution of 4-(4-nitrobenzyl)pyridine in acetone (1 in 20) and evaporate on a water-bath to dryness. Dissolve the residue in 1 mL of warmed methanol and add 2 drops of ammonia solution (28): a purple color develops.

(2) Dissolve 0.1 g of Melphalan in 10 mL of dilute sodium hydroxide TS and heat in a water-bath for 10 minutes. After cooling, add dilute nitric acid to acidify and filter: the filtrate responds to the Qualitative Tests for chloride.

(3) Determine the absorption spectra of the solutions of Melphalan and Melphalan RS in methanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) ***Ionizable chloride***—Weigh accurately about 0.5 g of Melphalan, dissolve in 80 mL of diluted nitric acid (1 in 40), stir for 2 minutes and titrate with 0.1 mol/L silver nitrate VS (potentiometric titration, Endpoint Detection method in Titrimetry): the consumed volume is not more than 1.0 mL to 0.50 g of Melphalan.

(2) ***Heavy metals***—Proceed with 1.0 g of Melphalan according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Melphalan according to Method 3 and perform the test (not more than 2 ppm).

Loss on Drying Not more than 7.0 % (1 g, in vacuum at a pressure not exceeding 0.67 kPa, 105 °C, 2 hours).

Residue on Ignition Not more than 0.3 % (1 g).

Nitrogen Content Weigh accurately about 325 mg of Melphalan, dissolve in 0.1 mol/L sulfuric acid TS and perform the test as directed under Nitrogen Determination: the amount of nitrogen (N: 14.01) is between 8.90 % and 9.45 %, calculated on the dried basis.

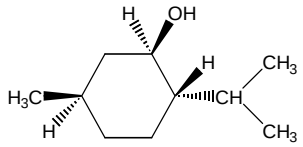
Assay Weigh accurately about 0.25 g of Melphalan, add 20 mL of a solution of potassium hydroxide (1 in 5) and heat under a reflux condenser in a water-bath for 2 hours. After cooling, add 75 mL of water and 5 mL of nitric acid, cool and titrate with 0.1 mol/L silver nitrate VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Make any necessary correction by using the results obtained in the Purity (1).

Each mL of 0.1 mol/L silver nitrate VS
= 15.260 mg of $C_{13}H_{18}Cl_{12}N_2O_2$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

dl-Menthol



and enantiomer

$C_{10}H_{20}O$: 156.27

(1*R*,2*S*,5*R*)- and (1*R*,2*S*,5*R*)-5-Methyl-2-propan-2-ylcyclohexan-1-ol [*89-78-1*]

dl-Menthol contains not less than 98.0 % and not more than 101.0 % of *dl*-menthol ($C_{10}H_{20}O$).

Description *dl*-Menthol appears as colorless crystals, and has characteristic and refreshing odor and burning taste, followed by a cool taste.
dl-Menthol sublimes gradually at room temperature.
dl-Menthol is very soluble in ethanol (95) or in ether and very slightly soluble in water.

Identification Proceed as directed in the Identification under *l*-Menthol.

Specific Optical Rotation $[\alpha]_D^{20}$: -2.0 ~ +2.0° (2.5 g, ethanol (95), 25 mL, 100 mm).

Congealing Point 27 ~ 28 °C.

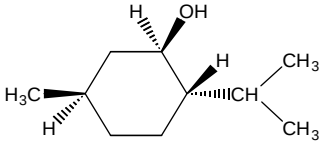
Purity Proceed as directed in the Purity under *l*-Menthol

Assay Proceed as directed in the Assay under *l*-Menthol

Each mL of 1 mol/L sodium hydroxide VS
= 156.27 mg of $C_{10}H_{20}O$

Containers and Storage ***Containers***—Tight containers.
Storage—In a cold place.

l-Menthol



and enantiomer

$C_{10}H_{20}O$: 156.27

(1*R*,2*S*,5*R*)-5-Methyl-2-propan-2-ylcyclohexan-1-ol [*2216-51-5*]

l-Menthol contains not less than 98.0 % and not more than 101.0 % of *l*-menthol ($C_{10}H_{20}O$).

Description *l*-Menthol appears as colorless crystals, and has characteristic and refreshing odor and burning taste, followed by a cool taste.
l-Menthol is very soluble in ethanol (95) or in ether and very slightly soluble in water.
l-Menthol sublimes gradually at room temperature.

Identification (1) Triturate *l*-Menthol with an equal amount of camphor, chloral hydrate or thymol: the mixture liquefies.

(2) Shake 1 g of *l*-Menthol with 20 mL of sulfuric acid: the mixture becomes turbid with a yellow-red color. Allow to stand for 3 hours: a clear, oily layer which possesses no aroma of menthol is separated.

Specific Optical Rotation $[\alpha]_D^{20}$: -45.0 ~ -51.0° (2.5 g, ethanol (95), 25 mL, 100 mm).

Melting Point 42 ~ 44 °C.

Purity (1) ***Non-volatile residue***—Volatilize 2.0 g of *l*-Menthol on a water-bath and dry the residue at 105 °C for 2 hours: the residue is not more than 1.0 mg.

(2) ***Thymol***—Add 0.20 g of *l*-Menthol to a cold mixture of 2 mL of acetic acid (100), 6 drops of sulfuric acid and 2 drops of nitric acid: no green to blue-green color immediately develops.

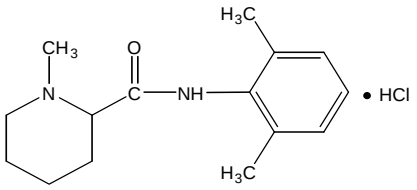
(3) ***Nitromethane or nitroethane***—Take 0.5 g of *l*-Menthol placed in a flask, add 2 mL of sodium hydroxide solution (1 in 2) and 1 mL of hydrogen peroxide (30), connect a reflux condenser to the flask and boil the mixture gently for 10 minutes. After cooling, add water to make exactly 20 mL and filter. Take 1 mL of the filtrate in a Nessler tube, add water to make 10 mL, neutralize with dilute hydrochloric acid, add another 1 mL of dilute hydrochloric acid and cool. To the mixture, add 1 mL of a solution of sulfanilic acid (1 in 100), allow to stand for 2 minutes and then add 1 mL of a solution of *N*-(1-naphthyl)-*N'*-diethyl-ehylenediamine oxalate (1 in 1000) and water to make 25 mL: no red-purple color immediately develops.

Assay Weigh accurately about 2.0 g of *l*-Menthol, add exactly 20 mL of a mixture of dehydrated pyridine and acetic anhydride (8 : 1), connect a reflux condenser and heat on a water-bath for 2 hours. Wash the condenser, with 20 mL of water and titrate with 1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS). Perform a blank determination and make any necessary correction

Each mL of 1 mol/L sodium hydroxide VS
= 156.27 mg of $C_{10}H_{20}O$

Containers and Storage ***Containers***—Tight containers.
Storage—In a cold place.

Mepivacaine Hydrochloride



$C_{15}H_{22}N_2O \cdot HCl$: 282.81

N-(2,6-Dimethylphenyl)-1-methylpiperidine-2-carboxamide hydrochloride [*1722-62-9*]

Mepivacaine Hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of mepivacaine hydrochloride ($C_{15}H_{22}N_2O \cdot HCl$).

Description Mepivacaine Hydrochloride appears as white crystals or crystalline powder.
Mepivacaine Hydrochloride is freely soluble in water or in methanol, soluble in acetic acid (100) and sparingly soluble in ethanol (99.5).
A solution of Mepivacaine Hydrochloride (1 in 10) shows no optical rotation.

Melting point—About 256 °C (with decomposition).

Identification (1) Determine the absorption spectra of the solutions of Mepivacaine Hydrochloride and Mepivacaine Hydrochloride RS (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Mepivacaine Hydrochloride and Mepivacaine Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Mepivacaine Hydrochloride (1 in 50) responds to the Qualitative Tests for chloride.

pH Dissolve 0.2 g of Mepivacaine Hydrochloride in 10 mL of water: the pH of this solution is between 4.0 and 5.0.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Mepivacaine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) ***Sulfate***—Perform the test with 0.5 g of Mepivacaine Hydrochloride. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038 %).

(3) ***Heavy metals***—Proceed with 2.0 g of Mepivacaine Hydrochloride according to Method 1 and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 5 ppm).

(4) ***Related substances***—Dissolve 0.10 g of Mepivacaine Hydrochloride in 5 mL of methanol and use this solution as the test solution. Pipet 1.0 mL of the test solution and add methanol to make exactly 20 mL. Pipet 2 mL of this solution, add methanol to make exactly 50 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ether, methanol and ammonia solution (28) (100 : 5 : 1) to a distance of about 10 cm and air-dry the plate. Spray evenly bismuth nitrate-potassium iodide TS on the plate: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

(5) ***2,6-Dimethylaniline***—Weigh accurately about 5.0 g of Mepivacaine Hydrochloride and dissolve in methanol to make exactly 10 mL. Pipet 2 mL of this solution, add 1 mL of a solution of 4-dimethylaminobenzaldehyde in methanol (1 in 100) and 2 mL of acetic acid (100), allow to stand for 10 minutes and use this solution as the test solution. Separately, weigh accurately about 5.0 mg of 2,6-dimethylaniline and dissolve in methanol to make exactly 10 mL. Pipet 1 mL of this solution and add methanol to make exactly 100 mL. Proceed with 2 mL of this solution in the same manner as the test solution and use this solution as the standard solution. The test solution has no more color than the standard solution at the same time (not more than 100 ppm).

Loss on Drying Not more than 1.0 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.4 g of Mepivacaine Hydrochloride, previously dried, dissolve in 10 mL of acetic acid (100) and add 70 mL of acetic anhydride. Titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 28.281 mg of $C_{15}H_{22}N_2O \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Mepivacaine Hydrochloride Injection

Mepivacaine Hydrochloride Injection is an aqueous solution for injection. Mepivacaine Hydrochloride Injection contains not less than 95.0 % and not more than

105.0 % of the labeled amount of mepivacaine hydrochloride ($C_{15}H_{22}N_2O \cdot HCl$: 282.81).

Method of Preparation Prepare as directed under Injections, with Mepivacaine Hydrochloride.

Description Mepivacaine Hydrochloride Injection is a clear, colorless liquid.

pH—4.5 ~ 6.8.

Identification Take a volume of Mepivacaine Hydrochloride Injection, equivalent to 20 mg of Mepivacaine Hydrochloride according to the labeled amount, add 1 mL of sodium hydroxide TS and extract with 20 mL of hexane. To 8 mL of the hexane extract, add 20 mL of 1 mol/L hydrochloric acid TS, shake vigorously and determine the absorption spectrum of the water layer as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 261 nm and 265 nm and between 270 nm and 273 nm.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.6 EU/mg of mepivacaine hydrochloride.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Iinjection It meets the requirement.

Determination of Volume of Injection in Container It meets the requirement.

Assay Take an exactly measured volume of Mepivacaine Hydrochloride Injection, equivalent about 40 mg of Mepivacaine Hydrochloride according to the labeled amount, add 4.0 mL of the internal standard solution and 0.001 mol/L hydrochloric acid TS to make exactly 20 mL and use this solution as the test solution. Separately, weigh accurately about 40 mg of Mepivacaine Hydrochloride RS, previously dried at 105 °C for 3 hours, dissolve in 0.001 mol/L hydrochloric acid TS, add 4.0 mL of the internal standard solution and 0.001 mol/L hydrochloric acid TS to make exactly 20 mL and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of mepivacaine hydrochloride to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of mepivacaine hydrochloride } (C_{15}H_{22}N_2O \cdot HCl) = \text{Amount (mg) of Mepivacaine Hydrochloride RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of benzophenone in methanol (1 in 4000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

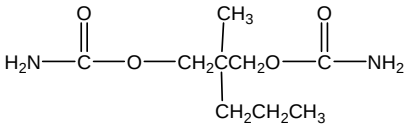
Mobile phase: Dissolve 2.88 g of sodium lauryl sulfate in 1000 mL of a mixture of 0.02 mol/L phosphate buffer solution, pH 3.0 and acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of mepivacaine is about 6 minutes.

Selection of column: When the procedure is run with 5 μL of a standard solution under the above operating conditions, mepivacaine and benzophenone are eluted in this order with the resolution between their peaks being not less than 6.0.

Containers and Storage ***Containers***—Hermetic containers.

Meprobamate



Methylphenidate Hydrochloride $C_9H_{18}N_2O_4$: 218.25

[2-(Carbamoyloxymethyl)-2-methylpentyl] carbamate [*57-53-4*]

Meprobamate contains not less than 97.0 % and not more than 101.0 % of meprobamate ($C_9H_{18}N_2O_4$), calculated on the dried basis.

Description Meprobamate appears as white powder and has a characteristic odor and a bitter taste.

Meprobamate is slightly soluble in water, freely soluble in acetone or in ethanol (95) and sparingly soluble in ether.

Identification (1) Determine the infrared spectra of Meprobamate and Meprobamate RS, previously dried, as directed in the potassium bromide disk method under Infrared spectrophotometry: both spectra exhibit the similar intensities of absorption at the same wavenumbers. If the maximum absorption wavenumbers are different between Meprobamate and Meprobamate RS, dissolve Meprobamate and Meprobamate RS in acetone to make solution containing 8 mg per mL, mix 0.1 mL of this solution with 1 mL of n-heptane and evapo-

rate the solvent below 30 °C under nitrogen, respectively. Dry the residues in vaccum at room temperature for 30 minutes and repeat the test.

(2) Perform the test as directed under the related substances: spots from the test solution and the standard solution exhibit the same R_f value and color.

Melting Point 103 ~ 107 °C. The difference between the beginning and the end temperature of melting is not more than 2 °C.

Purity (1) ***Heavy metals***—Dissolve 2.0 g of Meprobamate in a mixture of water and acetone (15:85) to make 20 mL. Use this solution as the test solution. Separately, to 2.0 mL of standard lead solution, add a mixture of water and acetone (15:85) to make 20 mL. To 10 mL of this solution, add 2 mL of the test solution and use this solution as the control solution. Separately, to 10 mL of a mixture of water and acetone (15:85), add 2 mL of the test solution and use this solution as the blank solution. To 12 mL each of the test solution, the control solution and the blank solution, add 2 mL of pH 3.5 acetate buffer, mix, add 1.2 mL of thioacetamide TS and mix immediately. Allow to stand for 2 minutes: the test solution has no more color than the control solution (not more than 10 ppm).

System suitability: The control solution shows a faint brown color compared to the blank solution.

(2) ***Related substances***—Weigh accurately a portion of Meprobamate, dissolve in ethanol (95) to make a solution containing 0.1 g per mL and use this solution as the test solution. Add ethanol (95) to the test solution and use this solution as the identification solution to make a solution containing 1.0 mg per mL. Separately, weigh accurately a volume of Meprobamate RS, previously dried at 60 °C for 3 hours under vaccum and dissolve in ethanol (95) to make a solution containing 1.0 mg per mL. Add ethanol (95), dilute quantitatively and use this solution as the standard solution.

Standard solutions	Dilution	Concentration (mg/mL)	Ratio (%) of the sample
A	No dilution	1.0	1.0
B	4 in 5	0.8	0.8
C	3 in 5	0.6	0.6
D	2 in 5	0.4	0.4
E	1 in 5	0.2	0.2

Perform the test with the test solution and the standard solutions as directed under the Thin-layer Chromatography. Spot 2 μL each of the test solution, the identification solution and the standard solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, acetone and pyridine (7 : 3 : 1) to a distance of about 15 cm, air-dry the plate for 15 minutes, heat at 100 °C for 15 minutes and cool. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 110 °C for 15 to 20 minutes, cool and allow to stand at room temperature until the spot of blue-purple color is observed. It usually takes 30 to 60 minutes to develop color. The R_f value of the principal spot from the standard solution and the spots other than the principal spot from the test solution are not larger or not more intense than the spot from the standard solution A (not more than 1.0 %). Total intensity of all the spots other than the principal spot from the test solution is not more than 2.0 %.

(3) ***Methyl carbamate***: Weigh accurately about 1.0 g of finely powdered Meprobamate, transfer to a beaker, add 5.0 mL of water and mix well. Filter the solution through a funnel with glass wool and use the filtrate as the test solution. Separately, weigh accurately a volume of Methyl Carbamate RS, previously dried in vacuum at 60 °C for 3 hours, add water to make a solution containing 1.0 mg per mL and use this solution as the standard solution. Perform the test with 50 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions: the peak area of methyl carbamate from the test solution is not larger than that from the standard solution (not more than 0.5 %).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 200 nm).

Column: A stainless steel column, about 3.9 mm to 4.6 mm in internal diameter and 25 cm to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm to 10 μm in particle diameter).

Mobile phase: Water.

Flow rate: 1 mL/minute.

System suitability

System repeatability: When the test is repeated 6 times with 20 μL each of standard solution under the above operating conditions: the relative standard deviation of the peak area of methyl carbamate is not more than 2.0 %.

Loss on Drying Not more than 0.5 % (1.0 g, 60 °C, in vacuum, 3 hours).

Assay Weigh accurately about 0.4 g of Meprobamate in an Erlenmeyer flask, add 40 mL of hydrochloric acid and a few boiling stones and reflux at the boiling temperature for 90 minutes. Boil the solution without the refluxing apparatus to make 5 mL to 10 mL, cool, add 50 mL of water and 1 drop of methyl red TS and neutralize the solution with 10 mol/L of sodium hydroxide TS carefully until the color of indicator changes. If necessary, add 1 mol/L of hydrochloric acid until the color of the solution changes to red and titrate with 0.1 mol/L of sodium hydroxide VS carefully, again. Add a mixture of 15 mL of formaldehyde TS and 15 mL of water previously neutralized with phenolphthalein TS and titrate with 0.1 mol/L sodium hydroxideVS until a yellow color develops. Again, add 0.2 mL of phenolphthalein TS and titrate with 0.1 mol/L of sodium hy-

droxide until red color is observed. Perform a blank determination and make any necessary correction.

Each mL of the total volmne 0.1 mol/L sodium hydroxide TS consumed after the addition of formaldehyde TS = 10.912 mg of $C_9H_{18}N_2O_4$

Containers and Storage ***Containers***—Tight containers.

Meprobamate Tablets

Meprobamate Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of meprobamate ($C_9H_{18}N_2O_4$: 218.25).

Method of Preparation Prepare as directed under Tablets, with Meprobamate.

Identification (1) Take a volume of powdered Meprobamate Tablets, equivalent to 0.8 g of Meprobamate according to the labeled amount, add 5 mL of dehydrated ethanol, shake frequently and heat for 5 minutes until it starts to boil. After cooling, filter, add 15 mL of hexane and mix by shaking. Filter by suction and dry the precipitate (crystal) at 60 °C: Melting temperature of the crystals is between 103 °C and 107 °C. The difference between the beginning and the end temperatures of melting is not more than 2 °C.

(2) Proceed with the crystals as directed in the Identification (1) under Meprobamate.

Dissolution Test Perform the test with 1 tablet of Meprobamate Tablets at 100 revolutions per minute according to the Method 1 under Dissolution Test, using 900 mL of water as the dissolution solution. Pipet 30.0 mL of dissolved solution after 30 minutes from the start of the test and perform the test with the filtrate as directed in the Assay.
Dissolution rate of Meprobamate Tablets in 30 minutes is not less than 75 %.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Meprobamate Tablets. Weigh accurately a portion of the powder, equivalent to about 0.25 g of meprobamate ($C_9H_{18}N_2O_4$), dissolve in 15 mL of acetonitrile by shaking, add water to make exactly 50 mL and filter. Discard the first 10 mL of the filtrate and use the subsequent filtrate as the test solution. Separately, weigh accurately about 50 mg of Meprobamate RS, dissolve in 3 mL of acetonitrile by shaking, add water to make exactly 10 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the peak areas, A_T and A_S, of the test solution and the standard solution, respectively.

$$\text{Amount (mg) of meprobamate } (C_9H_{18}N_2O_4) = \text{Amount (mg) of Meprobamate RS} \times \frac{A_T}{A_S} \times 5$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 200 nm).

Column: A stainless steel column, about 3.9 mm to 4.6 mm in internal diameter and 25 cm to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm to 10 μm in particle diameter).

Mobile phase: A mixture of water and acetonitrile (7 : 3).

Flow rate: 1 mL/minute.

System suitability

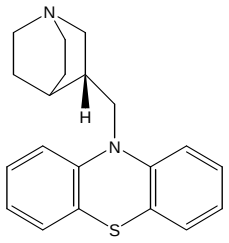
System performance: Dissolve 25 mg of Meprobamate in 1 mL of acetonitrile, shake and add 1 mL of phenacetin solution and water to make 5 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, meprobamate and phenacetin is eluted in this order with the resolution between these peaks being not less than 2.0. The peak area of phenacetin is 65.0 to 100.0 % of that of meprobamate.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of meprobamate is not more than 2.0 %.

Phenacetin solution—Dissolve 25 mg of phenacetin in acetonitrile to make 200 mL. Pipet 20 mL of this solution and add 30 mL of acetonitrile and water to make 100 mL.

Containers and Storage ***Containers***—Well-closed containers.

Mequitazine



and enantiomer

$C_{20}H_{22}N_2S$: 322.47

(*RS*)-10-(1-Azabicyclo[2.2.2]octan-3-ylmethyl)phenothiazine [*29216-28-2*]

Mequitazine, when dried, contains not less than 98.5 % and not more than 101.0 % of mequitazine ($C_{20}H_{22}N_2S$).

Description Meauitazine appears as white crystals or crystalline powder.
Meauitazine is freely soluble in methanol or in acetic acid (100), soluble in ethanol (95), and practically insoluble in water.
A solution of Meauitazine in methanol(1 in 50) shows no optical rotation.
Meauitazine is gradually colored by light.

Identification (1) Determine the absorption spectra of solutions of Mequitazine and Mequitazine RS in ethanol (95) (1 in 250000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Mequitazine and Mequitazine RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 146 ~ 150 °C.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Meauitazine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Perform the test without exposure to light, using light-resistant vessels. Dissolve 50 mg of Meauitazine in 5 mL of methanol, and use this solution as the test solution. Pipet 1.0 mL of the test solution, add methanol to make exactly 50 mL, pipet 5.0 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethylacetate, methanol and diethylamine (7 : 2 : 2) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the number of the spots other than the principal spot from the sample solution is not more than 3 and they are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, P_2O_5, 60 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

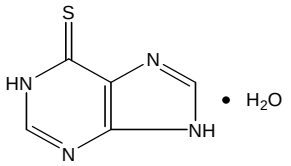
Assay Weigh accurately about 0.25 g of Meauitazine, previously dried, dissolve in 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.247 mg of $C_{20}H_{22}N_2S$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Mercaptopurine Hydrate



Mercaptopurine $C_5H_4N_4S \cdot H_2O$:170.19

3,7-Dihydropurine-6-thione hydrate [*6112-76-1*]

Mercaptopurine Hydrate contains not less than 98.0 % and not more than 101.0 % of mercaptopurine ($C_5H_4N_4S$: 152.18), calculated on the anhydrous basis.

Description Mercaptopurine Hydrate appears as pale yellow to yellow crystals or crystalline powder and is odorless.
Mercaptopurine Hydrate is practically insoluble in water, in acetone or in ether.
Mercaptopurine Hydrate dissolves in sodium hydroxide TS or in ammonia TS.

Identification (1) Dissolve 0.6 g of Mercaptopurine Hydrate in 6 mL of sodium hydroxide solution (3 in 100) and add slowly 0.5 mL of iodomethane with vigorous stirring. Stir well for 10 minutes, cool in an ice-bath and adjust the pH to about 5 by dropping acetic acid. Collect the separated crystals by filtration, recrystallize from water and dry at 120 °C for 30 minutes: the crystals melt between 218 °C and 222 °C (with decomposition).

(2) Determine the absorption spectra of the solutions of Mercaptopurine Hydrate and Mercaptopurine Hydrate RS in 0.1 mol/L hydrochloric acid VS (1 in 200000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) ***Clarity of solution***—Dissolve 0.20 g of Mercaptopurine Hydrate in 10 mL of ammonia TS: the solution is clear.

(2) ***Sulfate***—Dissolve 50 mg of Mercaptopurine Hydrate in 10 mL of dilute hydrochloric acid, add 5 drops of barium chloride TS and allow to stand for 5 minutes: no turbidity is produced.

(3) ***Heavy metals***—Proceed with 1.0 g of Mercap-

topurine Hydrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Hypoxanthine***—Dissolve 50 mg of Mercaptopurine Hydrate in exact 10 mL of a solution of ammonia solution (28) in methanol (1 in 10) and use this solution as the test solution. Separately, dissolve 5.0 mg of hypoxanthine in exact 100 mL of a solution of ammonia solution (28) in methanol (1 in 10) and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, chloroform, n-butyl formate and ammonia solution (28) (8 : 6 : 4 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the test solution is not larger than and not more intense than that from the standard solution.

(5) ***Phosphorus***—Take 0.20 g of Mercaptopurine Hydrate in a crucible, add 2 mL of diluted sulfuric acid (3 in 7), then heat gently, slowly adding drop-wise several 0.5 mL volumes of nitric acid, until the liquid becomes colorless. Continue to heat until most of the liquid has evaporated, cool and dissolve the residue in 10 mL of water. Transfer the solution to a 25 mL volumetric flask, wash the crucible with two 4 mL volumes of water, combine the washings with the solution in the volumetric flask and use this solution as the test solution. Separately, dissolve 0.4396 g of monobasic potassium phosphate in water to make exactly 200 mL. To 2.0 mL of this solution, add water to make exactly 100 mL. Transfer 2.0 mL of this solution to a 25 mL volumetric flask, add 16 mL of water and use this solution as the standard solution. To the test solution and the standard solution, add 1 mL of diluted sulfuric acid (3 in 7), 0.5 mL of nitric acid, 0.75 mL of ammonium molybdate TS, 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS and water to make 25 mL and allow to stand for 5 minutes. Perform the test with the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry, using water as the blank: the absorbance of the subsequent solution of the test solution at 750 nm is not larger than that of the subsequent solution of the standard solution.

Water 10.0 ~ 12.0 % (0.2 g, volumetric titration, back titration).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.25 g of Mercaptopurine Hydrate, dissolve in 90 mL of *N,N*-dimethylformamide and titrate with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration, Endpoint Detection Methods in Titrimetry). Perform a blank determination with a mixture of 90 mL of *N,N*-dimethylformamide and 15 mL of water and make any necessary correction.

Each mL of 0.1 mol/L
tetramethylammonium hydroxide VS
= 15.218 mg of $C_5H_4N_4S$

Containers and Storage ***Containers***—Well-closed containers.

Meropenem for Injection

Meropenem for Injection is a preparation for injection, which is dissolved before use. Meropenem for Injection contains not less than 93.0 % and not more than 107.0 % of the labeled amount of meropenem ($C_{17}H_{25}N_3O_5S$: 383.47).

Method of Preparation Prepare as directed under Injections, with Meropenem Hydrate and Sodium Carbonate.

Description Meropenem for Injection appears as white to pale yellow crystalline powder.

Identification Determine the infrared spectrum of Meropenem for Injection as directed in the potassium bromide disk method under Infrared Spectrophotometry: it exhibits absorption at the wavenumbers of about 3410 cm^{-1}, 1750 cm^{-1}, 1655 cm^{-1}, 1583 cm^{-1} and 1391 cm^{-1}.

pH The pH of a solution obtained by dissolving an amount of Meropenem for Injection, equivalent to 0.25 g (potency) of meropenem, in 5 mL of water is between 7.3 and 8.3.

Purity (1) ***Color of solution***—Dissolve an amount of Meropenem for Injection, equivalent to 1.0 g (potency) of Meropenem Hydrate according to the labeled amount, in 20 mL of water: the solution has no more color than the following control solution.

Control solution—To 0.3 mL of cobalt (II) chloride hexahydrate stock CS and 1.2 mL of iron (III) chloride hexahydrate stock CS, add 18.5 mL of diluted hydrochloric acid (1 in 40).

(2) ***Related substances***—Weigh accurately about 0.125 g (potency) of Meropenem for Injection, dissolve in triethylamine-phosphate buffer (pH 5.0) to make exactly 25 mL and use this solution as the test solution (prepare before use). Separately, weigh accurately about 25 mg (potency) of Meropenem RS and dissolve in triethylamine-phosphate buffer (pH 5.0) to make exactly 10 mL. Pipet 1 mL of this solution, add triethylamine-phosphate buffer (pH 5.0) to make exactly 200 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed in Purity (2) under

Meropenem. Determine the peak area of the ring-opened meropenem and dimer from the test solution and the peak area of meropenem from the standard solution, and calculate the amount of the ring-opened meropenem and dimer according to the following equations (not more than 0.5 % for the ring-opened meropenem, not more than 0.5 % for the dimer).

$$\text{Amount (\%) of the ring-opened meropenem} = \frac{A_{Ta}}{A_S} \times \frac{\text{Amount[mg (potency)]of Meropenem RS}}{\text{Amount (mg) of Meropenem for Injection taken}} \times \frac{5}{4}$$

$$\text{Amount (\%) of the dimer} = \frac{A_{Tb}}{A_S} \times \frac{\text{Amount[mg (potency)]of Meropenem RS}}{\text{Amount (mg) of Meropenem for Injection taken}} \times \frac{5}{4}$$

A_{Ta}: Peak area of the ring-opened meropenem in the test solution

A_{Tb}: Peak area of the dimer in the test solution

A_S: Peak area of meropenem in the standard solution

Loss on Drying 9.5 ~ 12.0 % (0.1 g, 0.7 kPa, 60 °C, 3 hours).

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.12 EU/mg (potency) of meropenem.

Foreign Insoluble Matter Test It meets the requirement.

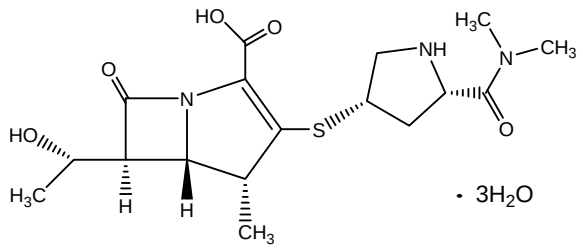
Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Perform the test as directed in the Assay under Meropenem Hydrate. Weigh accurately an amount of Meropenem for Injection, equivalent to about 50 mg (potency) according to the labeled potency, add exactly 10 mL of the internal standard solution, add triethylamine-phosphate buffer (pH 5.0) to make 100 mL and use this solution as the test solution.

Containers and Storage ***Containers***—Hermetic containers.

Meropenem Hydrate



$C_{17}H_{25}N_3O_5S \cdot 3H_2O$: 437.51

(4*R*,5*S*,6*S*)-3-[(3*S*,5*S*)-5-(Dimethylcarbamoyl)pyrrolidin-3-yl]sulfanyl-6-[(1*S*)-1-hydroxy-ethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid trihydrate [*119478-56-7*]

Meropenem Hydrate contains not less than 980 μg (potency) and not more than 1010 μg (potency) per mg of meropenem ($C_{17}H_{25}N_3O_5S$: 383.47), calculated on the anhydrous basis.

Description Meropenem Hydrate appears as white to pale yellow crystalline powder.
Meropenem Hydrate is sparingly soluble in water and practically insoluble in ethanol (95) or in ether.
Meropenem Hydrate dissolves in sodium hydrogen carbonate TS.

Identification (1) Dissolve 10 mg (potency) of Meropenem Hydrate in 2 mL of water, add 3 mL of hydroxylamine hydrochloride-ethanol TS, allow to stand for 5 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS and shake: a red-brown color develops.

(2) Determine the absorption spectra of the solutions of Meropenem Hydrate and Meropenem RS in water (3 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Meropenem Hydrate and Meropenem RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: -17 ~ -21° (0.220 g calculated on the anhydrous basis, water, 50m L, 100 mm).

pH The pH of a solution obtained by dissolving 0.1 g (potency) of Meropenem Hydrate in 10 mL of water is between 4.0 and 6.0.

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Meropenem Hydrate in 10 mL of sodium hy-

drogen carbonate TS: the solution is clear and has no more color than the following control solution.

Control solution—Pipet 0.3 mL of cobalt (II) chloride hexahydrate stock CS and 1.2 mL of iron (III) chloride stock CS and add 18.5 mL of diluted hydrochloric acid (1 in 40).

(2) ***Heavy metals***—Proceed with 2.0 g of Meropenem Hydrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Weigh accurately about 50 mg (potency) of Meropenem Hydrate, dissolve in triethylamine-phosphate buffer (pH 5.0) to make exactly 10 mL and use this solution as the test solution. Prepare the test solution before use. Pipet 1 mL of this solution and add triethylamine-phosphate buffer (pH 5.0) to make exactly 100 mL. Pipet 3 mL of this solution, add triethylamine-phosphate buffer (pH 5.0) to make exactly 10 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine each peak area in each solution by the automatic integration method: the peak area of the ring-opened meropenem having the relative retention time of about 0.5 and the peak area of the dimer having the relative retention time of 2.2 with respect to meropenem obtained from the test solution are not larger than the peak area of meropenem from the standard solution, and the area of the peak other than meropenem, the ring-opened meropenem and the dimer from the test solution is not larger than 1/3 times the peak area of meropenem from the standard solution. The total area of peaks other than meropenem from the test solution is not larger than 3 times the peak area of meropenem from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column about 6.0 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: To 1000 mL of triethylamine-phosphate buffer (pH 5.0), add 70 mL of acetonitrile and mix.

Flow rate: Adjust the flow rate so that the retention tie of meropenem is about 6 minutes.

System suitability

Test for required detectability: Pipet 5 mL of the standard solution and add triethylamine-phosphate buffer (pH 5.0) to make exactly 25 mL. Confirm that the peak area of meropenem obtained from 10 μL of this solution is equivalent to 16 % to 24 % of that from the standard solution.

System performance: Warm the test solution at 60 °C for 30 minutes. When the procedure is run with 10 μL of this solution under the above operating conditions, the ring-opened meropenem, meropenem and dimer are eluted in this order with the resolution between the peaks of the ring-opened meropenem and meropenem being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of meropenem is not more than 1.5 %.

Time span of measurement: About 7 times as long as the retention time of meropenem.

Water 11.4 ~ 13.4 % (0.1 g, volumentric titration, direct titration).

Residue on Ignition Not more than 0.10 % (1 g).

Sterility Test It meets the requirement, when Meropenem Hydrate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.12 EU/mg (potency) of meropenem, when Meropenem Hydrate is used in a sterile preparation.

Assay Weigh accurately about 50 mg (potency) each of Meropenem Hydrate and Meropenem RS, add exactly 10 mL of the internal standard solution, dissolve in triethylamine-phosphate buffer (pH 5.0) to make 100 mL and use these solutions as the test solution and the standard solution. Perform the test with 5 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the ratios, Q_T and Q_S, of the peak area of meropenem to that of the internal standard in the test solution and the standard solution.

$$\text{Amount [μg (potency)] of meropenem } (C_{17}H_{25}N_3O_5S) = \text{Amount [μg (potency)] of Meropenem RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—Dissolve 1.0 mL of benzyl alcohol in triethylamine-phosphate buffer (pH 5.0) to make 300 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column about 6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: A mixture of triethylamine-phosphate buffer (pH 5.0) and methanol (5:1).

Flow rate: Adjust the flow rate so that the retention time of Meropenem RS is about 7 minutes.

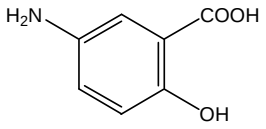
System suitability

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, meropenem and the internal standard are eluted in this order with the resolution between these peaks being not less than 20.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of meropenem to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Mesalazine



$C_7H_7NO_3$: 153.14

5-Amino-2-hydroxybenzoic acid [*89-57-6*]

Mesalazine contains not less than 98.5 % and not more than 101.0 % of mesalazine ($C_7H_7NO_3$), calculated on the dried basis.

Description Mesalazine appears as white or light grey or light red powder or crystals.
Mesalazine is very slightly soluble in water, and practically insoluble in ethanol (95).
Mesalazine dissolves in dilute sodium hydroxide solution and in dilute hydrochloric acid solution.

Identification (1) Dissolve 50 mg each of Mesalazine and Mesalazine RS in 10 mL of 1.03 % hydrochloric acid to make 100 mL. Dilute 5 mL of these solutions with 1.03 % hydrochloric acid to 200 mL. Determine the absorption spectra of both solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Mesalazine and Mesalazine RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Dissolve 50 mg each of Mesalazine and Mesalazine RS in 10 mL of a mixture of water and acetic acid (100) (1:1), dilute with methanol to 20 mL, and use these solutions the test solution and the standard solution, respectively. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography, develop with a mixture of 4-methyl-2-pentanone, methanol and acetic acid (100) (50 : 40 : 10) to a distance of about 10 cm, and dry the plate in air. Examine in ultraviolet light at 365 nm. The principal spot from the test solution corresponds to that from the standard solution.

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Mesalazine in 1 mol/L hydrochloric acid TS to make 20 mL, maintain the solution at 40 °C: the solution is clear. Maintaining the solution at 40 °C, measure the absorbance of the solution at 440 nm and 650 nm. The absorbance is not greater than 0.15 at 440 nm and 0.10 at 650 nm.

(2) ***Reducing substances***—Dissolve 0.10 g of Mesalazine in dilute hydrochloric acid to make 25 mL, add 0.2 ml of starch solution TS and 0.01 mol/L iodine TS, and allow to stand for 2 min: the solution is blue or purple-brown.

(3) ***Chlorides***— Dissolve 1.50 g of Mesalazine in 50 ml of formic acid, add 100 mL of water and 5 mL of 2 mol/L nitric acid, and titrate with 0.005 mol/L silver nitrate VS (potentiometric titration, Endpoint Detection Method in Titrimetry) (not more than 0.1 %).

Each mL of 0.005 mol/L silver nitrate VS
= 0.1773 mg of Cl

(4) ***Sulfates***—Shake 1.0 g of Mesalazine with 20 mL of water for 1 min and filter. Rinse the filter paper and residues on the filter paper. Combine the filtrate all together, dilute with water to make 40 mL, and use this solution as the test solution. Prepare the control solution with 0.42 mL of 0.005 mol/L sulfuric acid VS (not more than 0.02 %).

(5) ***Heavy metals*** —Proceed with 1.0 g of Mesalazine according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(6) ***Related substances I and II***—Dissolve 50.0 mg of Mesalazine in mobile phase A to make 50.0 mL, and use this solution as the test solution. Dissolve 5.0 mg of 2-aminophenol (related substance I) in mobile phase A to make 100 mL, and use this solution as the standard solution (1). Dissolve 5.0 mg of 4-aminophenol (related substance II) in mobile phase A to make exactly 250 mL. Pipet 1.0 mL of this solution, combine with 1.0 mL of the standard solution (1), and dilute with mobile phase A to make 100 mL. Use this solution as the standard solution (2). Pipet 1 mL of the test solution, dilute with mobile phase A to make 200 mL. Pipet 5 mL of this solution, and mix with 5 mL of the standard solution (1). Use this solution as the standard solution (3). Perform the test with 20 μL each of the test solution, the standard solution (2), and the standard solution (3) as directed under Liquid Chromatography according to the following conditions. Determine the peak areas by the automatic integration method. The peak area of the related substance II from the test solu-

tion is not more than that from the standard solution (2) (not more than 0.02 %), and the peak area of the related substance I from the test solution is not more than 4 times that from the standard solution (2) (not more than 0.02 %).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column about 4.6 mm internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Use variable mixtures of mobile A and mobile phase B, and program the chromatograph as follows.

Mobile phase A: Dissolve 2.2 g of perchloric acid and 1.0 g of phosphoric acid in water to make 1000 mL.

Mobile phase B: Dissolve 1.7 g of perchloric acid and 1.0 g of phosphoric acid in acetonitrile to make 1000 mL.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0-8	100	0
8-25	100→40	0→60
25-30	40→100	60→0
30-40	100	0

Flow rate: 1.0 mL/min.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution (3) according to the above operating conditions, the relative retention times are about 0.5 for the related substance II, 0.9 for the related substance I and 1.0 for Mesalazine, respectively. The resolution between the peaks of the related substance I and Mesalazine is not less than 3.0.

(7) ***Related substance IV*—** Dissolve 40.0 mg of Mesalazine in the mobile phase to make exactly 20 mL, and use this solution as the test solution. Dissolve 27.8 mg of aniline hydrochloride (related substance IV) in the mobile phase to make 100 mL. Pipet 0.2 mL of this solution, dilute with mobile phase to 20 mL, and use this solution as the standard solution. Perform the test with 50 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine the peak areas by the automatic integration method. The peak area of the related substance IV from the test solution is not more than that from the standard solution (0.001 %).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 205 nm).

Column: A stainless steel column about 4.0 mm internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: A mixture of phosphate buffer at pH 8.0 and methanol (85:15).

Flow rate: 1 mL/min.

System suitability

System performance: When the procedure is run with 50 μL of the standard solution according to the above operating conditions, the retention time of the related substance IV is about 15 minutes, and the signal-to-noise ratio is not less than 10.

Phosphate buffer at pH 8.0—Dissolve 1.41 g of potassium dihydrogen phosphate and 0.47 g of disodium hydrogen phosphate dihydrate in water to make 1000 mL. Adjust the solution pH to 8.0 with 1 mol/L sodium hydroxide solution.

(8) ***Related substances*—** Use freshly prepared the test solution, the standard solution, and mobile phases. Dissolve 50.0 mg of Mesalazine in the mobile phase A to make 50.0 mL, and use this solution as the test solution. Pipet 1.0 mL of the test solution, dilute with the mobile phase A to make exactly 100 mL, and use this solution as the standard solution (1). Dissolve 5.0 mg of 3-aminobenzoic acid (related substance V) in the mobile phase A to make exactly 100 mL. Pipet 1.0 mL of this solution, dilute with the mobile phase A to 25 mL, and use this solution as the standard solution (2). Dissolve 5 mg of the related substance V in the mobile phase A to make exactly 100 mL. Pipet 1 mL of the test solution, dilute with the mobile phase A to make 50 mL, and use this solution as the standard solution (3). Dissolve 10.0 mg of the related substance III (3-aminophenol) in the mobile phase A to make exactly 100 mL. Dilute 1.0 mL to 50 mL with the mobile phase A, and use this solution as the standard solution (4). Dissolve 5.0 mg of 2,5-dihydroxybenzoic acid (related substance VI) in the mobile phase A, and dilute to 100 mL with the mobile phase A. Dilute 1.0 mL to 50 mL with the mobile phase A, and use this solution as the standard solution (5). Dissolve 15.0 mg of salicylic acid (related substance VII) in the mobile phase A, and dilute to 100 mL with mobile phase A. Dilute 1.0 mL to 50 mL with the mobile phase A, and use this solution as the standard solution (6). Use the mobile phase A as the blank solution. Perform the test with 10 μL each of the blank solution, the test solution, the standard solutions (1), (3), (4), (5), and (6) as directed under Liquid Chromatography according to the following conditions. Determine the peak areas by the automatic integration method. The peak area of the related substance III from the test solution is not more than that from the standard solution (4) (0.2 %); the peak area of the related substance V is not more than that from the standard solution (3) (0.1 %); the peak area of the related substance

VI is not more than that from the standard solution (5) (0.1 %); the peak area of the related substance VII is not more than that from the standard solution (6) (0.3 %); the peak area of any other related substances is not more than 0.1 times the principal peak area from the standard solution (1) (0.1 %); and the total peak area of all related substances is not more than the principal peak area from the standard solution (1) (1.0 %). Disregard not only any peak areas that are not more than 0.05 times the principal peak area from the standard solution (1) but also any peaks from the blank solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column about 4.6 mm internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Use variable mixtures of mobile A and mobile phase B, and program the chromatograph as follows.

Mobile phase A: Dissolve 2.2 g of perchloric acid and 1.0 g of phosphoric acid in water to make 1000 mL.

Mobile phase B: Dissolve 1.7 g of perchloric acid and 1.0 g of phosphoric acid in acetonitrile to make 1000 mL.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0-7	100	0
7-25	100→40	0→60
25-30	40→100	60→0
30-40	100	0

Flow rate: 1.25 mL/min.

System suitability

System performance: When the procedure is run with 10 μL each of the standard solutions (2), (3), (4), (5), and (6) according to the above operating conditions, the relative retention times with reference to mesalazine are 0.8 for the related substance III, 1.2 for the related substance V, 3.1 for the related substance VI, and 3.9 for the related substance VII. The ratio of the height above the baseline of the peak due to the related substance V to the height above the baseline of the lowest point of the curve separating this peak from the peak due to mesalazine is not less than 1.5.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, constant mass).

Residue on Ignition Not more than 0.2 % (1 g).

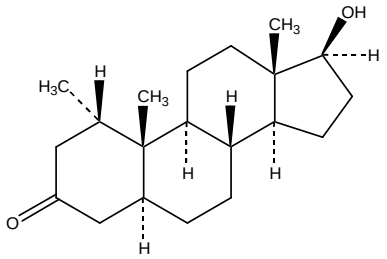
Assay Dissolve 50 mg of Mesalazine, accurately weighed, in 100 mL of boiling water. Cool rapidly to room temperature, and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry).

Each mL of 0.1 mol/L sodium hydroxide VS
= 15.31 mg of $C_7H_7NO_3$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Mesterolone



$C_{20}H_{32}O_2$: 304.47

(1*S*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*,17*S*)-17-Hydroxy-1,10,13-trimethyltetradecahydro-1*H*-cyclopenta[a]phenanthren-3(2*H*)-one [*1424-00-6*]

Mesterolone contains not less than 98.0 % and not more than 102.0 % of mesterolone ($C_7H_7NO_3$), calculated on the dried basis.

Description Mesterolone appears as white or pale yellow crystalline powder.
Mesterolone is sparingly soluble in acetone, in ethyl acetate and in methanol, and practically insoluble in water.

Identification Determine the infrared spectra of Mesterolone and Mesterolone RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 206 ~ 211 °C.

Specific Optical Rotation $[\alpha]_D^{20}$: + 20 ~ + 24° (0.2 g after drying, methylene chloride, 10 mL, 100 mm).

Purity (1) ***Related substances I***—Dissolve 0.1 g of Mesterolone in a mixture of methanol and dichloromethane (1 : 1) to make 10 mL, and use this solution as the test solution. Pipet 1 mL of the test solution, dilute to 200 mL with the mixture of methanol and dichloromethane (1 : 1), and use this solution as the standard solution (1). Dissolve 5 mg of 1α-methyl-5α-androstan-3β,17β-diol (related substance I) in the standard solution (1) to make 100 mL, and use this

solution as the standard solution (2). Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution, the standard solution (1), and the standard solution (2) on a plate of silica gel for thin-layer chromatography, develop with a mixture of toluene, acetone and methanol (85 : 15 : 2) to a distance of about 15 cm, and dry the plate in air. Examine in ultraviolet light at 366 nm, or spray with a 20 % toluenesulphonic acid in ethanol and heat the plate for 10 min at 120 °C. Any blue spots, excluding the principal spot, from the test solution is not more intense than the spot from the standard solution (1) (0.5 %).

(2) ***Other related substances***—Dissolve 50.0 mg of Mesterolone in a mixture of acetonitrile and water (4 : 1) to make 25 mL, and use this solution as the test solution. Separately, dissolve 50.0 mg of Mesterolone RS in a mixture of acetonitrile and water (4 : 1) to make 25 mL, and use this solution as the standard solution (1). Dissolve 10.0 mg of 17β-Hydroxy-1α-methyl-5α-androst-4-en-3-one (related substance II) in a mixture of acetonitrile and water (4 : 1) to make 5 mL, and use this solution as the standard solution (2). Combine 0.5 mL of the standard solution (1) and 0.5 mL of the standard solution (2), dilute with a mixture of acetonitrile and water (4 : 1) to 100 mL, and use this solution as the standard solution (3). Perform the test with 50 μL each of the test solution and the standard solution (3) as directed under Liquid Chromatography according to the following conditions. Determine the peak areas by the automatic integration method. The peak area of the related substance II from the test solution is not more than that of the related substance II from the standard solution (3) (0.5 %); the peak area of any other related substance is not more than half the peak area of mesterolone from the standard solution (3) (0.25 %); and the sum of total peak areas is not more than 1.5 times the peak area of mesterolone from the standard solution (3) (0.75 %). Disregard any peak areas that are not more than 0.1 times the peak area of mesterolone from the standard solution (3).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 200 nm)

Column: A stainless steel column about 4.6 mm internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Mobile phase: A mixture of methanol, water and acetonitrile (3:2:1).

Flow rate: 0.9 mL/min.

System suitability

System performance: Combine 0.5 mL of the standard solution (1) and 0.5 mL of the standard solution (2), and dilute with a mixture of acetonitrile and water (4 : 1) to 100 mL. When the procedure is run with 50 μL of this solution, the resolution between the peaks of mesterolone and the related substance II is not less than 6.0.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, constant mass).

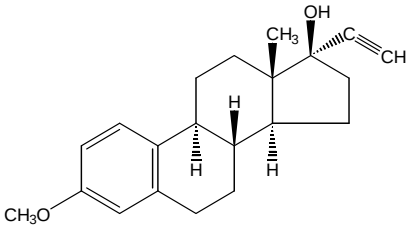
Residue on Ignition Not more than 0.1 % (1 g)

Assay Dissolve about 50 mg each of Mesterolone and Mesterolone RS, accurately weighed, in a mixture of acetonitrile and water (4 : 1) to make exactly 25 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed in the Liquid Chromatography according to the above operating conditions. Calculate the peak areas, A_T and A_S, of mesterolone of these solutions.

$$\text{Amount (mg) of mesterolone } (C_{20}H_{32}O_2) = \text{Amount (mg) of Mesterolone RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Tight containers.

Mestranol



$C_{21}H_{26}O_2$: 310.43

(8*R*,9*S*,13*S*,14*S*,17*R*)-17-Ethynyl-3-methoxy-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[a]phenanthren-17-ol [*72-33-3*]

Mestranol, when dried, contains not less than 97.0 % and not more than 102.0 % of mestranol ($C_{21}H_{26}O_2$).

Description Mestranol appears as white to pale yellow, crystalline powder and is odorless.
Mestranol is freely soluble in chloroform, soluble in 1,4-dioxane, sparingly soluble in ethanol (99.5) or in ether and practically insoluble in water.

Identification (1) Dissolve about 2 mg of Mestranol in 1 mL of a mixture of sulfuric acid and ethanol (99.5) (2 : 1): a red-purple color is observed with a yellow-green fluorescence.

(2) Determine the absorption spectra of solutions of Mestranol and Mestranol RS in ethanol (99.5) (1 in 10000) as directed under the Ultraviolte-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Mestranol and Mestranol RS, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +2 ~ +8° (0.2 g, after drying, 1,4-dioxane, 10 mL, 100 mm).

Melting Point 149 ~ 154 °C

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Mestranol according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Arsenic***—Prepare the test solution with 1.0 g of Mestranol according to Method 3 and perform the test (not more than 2 ppm).

(3) ***Related substances***—Dissolve 0.1 g of Mestranol in 20 mL of chloroform and use this solution as the test solution. Pipet 1.0 mL of the test solution, add chloroform to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (99.5) (29 : 1) to a distance of about 10 cm and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 5) on the plate and heat at 105 °C for 15 minutes: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (0.5 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (0.5 g).

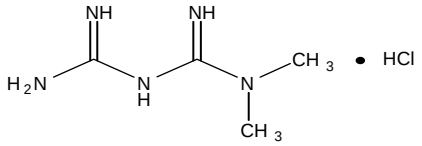
Assay Weigh accurately about 10 mg of each of Mestranol and Mestranol RS, previously dried, dissolve in ethanol (99.5) to make exactly 100 mL and use the test solution and the standard solution as the test solution and the standard solution, respectively. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 279 nm as directed under Ultraviolet-visible spectrophotometry.

$$\text{Amount (mg) of } C_{21}H_{26}O_2 = \text{Amount (mg) of Mestranol RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Metformin Hydrochloride



$C_4H_{11}N_5 \cdot HCl$: 165.63

3-(Diaminomethylidene)-1,1-dimethylguanidine hydrochloride [*1115-70-4*]

Metformin Hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$).

Description Metformin Hydrochloride appears as white crystals.
Metformin Hydrochloride is freely soluble in water, slightly soluble in ethanol (95) and practically insoluble in acetone or in dichloromethane.

Identification (1) Dissolve 20 mg each of Metformin Hydrochloride and Metformin Hydrochloride RS in water to make 5 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of water, 1-butanol and acetic acid (100) (50:40:10) to a distance of about 15 cm, and dry the plate at 100 °C to 105 °C for 15 minutes. Spray with a mixture of sodium nitroprusside solution (1 in 10), potassium ferricyanide TS and sodium hydroxide solution (1 in 10) (1 : 1 : 1). (This solution is made when used): the principal spot obtained from the test solution is the same in R_f value and color as the principal spot obtained from the standard solution.

(2) Determine the infrared spectra of Metformin Hydrochloride and Metformin Hydrochloride RS as directed in the potassium chloride disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Dissolve Metformin Hydrochloride in water (1 in 200): the solution responds to the Qualitative Tests for Chloride.

Melting Point 222 ~ 226 °C.

Purity (1) ***Clarity and color of solution***—Dissolve 2.0 g of Metformin Hydrochloride in 20 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Metformin Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Dissolve 0.50 g of Met-

formin Hydrochloride in a mobile phase to make exactly 100 mL, and use this solution as the test solution. Separately, dissolve 20.0 mg of cyanoguanidine in water to make exactly 100 mL. Pipet 1.0 mL of this solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution (1). Pipet 1.0 mL of the test solution, and add the mobile phase to make exactly 50 mL. And pipet 1.0 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution (2). Dissolve 10.0 mg of melamine in 90 mL of water, add 5 mL of the test solution and add water to make exactly 100 mL. Pipet 1.0 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution (3). Perform the test with 20 μL each of the test solution, the standard solution (1) and the standard solution (2) as directed under Liquid Chromatography according to the following conditions: in the chromatogram obtained with the test solution, the area of any peak corresponding to cyanoguanidine is not larger than the area of the peak obtained from the standard solution (1) (0.02 %), and the area of any peak other than the principal peak and any peak corresponding to cyanoguanidine is not larger than the area of the peak obtained from the standard solution (2) (0.1 %).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 218 nm).

Column: A stainless steel column, about 4.7 mm in internal diameter and about 11 cm in length, packed with benzenesulphonic acidized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Dissolve 17 g of ammonium dihydrogen phosphate in water to make 1000 mL. Adjust to the pH 3.0 with phosphoric acid.

Flow rate: 1 mL/minute.

System suitability

Detection sensitivity: Adjust the detection sensitivity so that the peak height of metformin hydrochloride obtained from 20 μL of the standard solution (1) is at least 50 % of the full scale.

System performance: When the procedure is run with 20 μL of the standard solution (3) under the above operating conditions, the resolution between peaks of melamine and metformin hydrochloride is not less than 10.

Time span of measurement: About twice as long as the retention time of Metformin Hydrochloride.

Loss on Drying Not more than 0.5 percent (1 g, 105 °C, 5 hours).

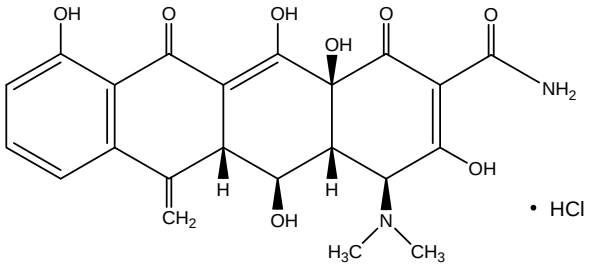
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 60 mg of Metformin Hydrochloride, dissolve in 4 mL of anhydrous formic acid, add 50 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 8.281 mg of $C_4H_{11}N_5 \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Methacycline Hydrochloride



$C_{22}H_{22}N_2O_8 \cdot HCl$: 478.88

(4*S*,4a*R*,5*S*,5a*R*,12a*R*)-4-(Dimethylamino)-1,5,10,11,12a-pentahydroxy-6-methylidene-3,12-dioxo-4,4a,5,5a-tetrahydrotetracene-2-carboxamide hydrochloride [*3963-95-9*]

Methacycline Hydrochloride contains not less than 832 μg (potency) per mg of methacycline ($C_{22}H_{22}N_2O_8$: 442.42).

Description Methacycline Hydrochloride appears as yellow crystalline powder or powder.
Methacycline Hydrochloride is soluble in water, sparingly soluble in methanol or in ethanol (95) and practically insoluble in chloroform or in ether.

Identification (1) Dissolve 20 mg (potency) of Methacycline Hydrochloride in 3 mL of water and add silver nitrate TS: a white turbidity is produced.

(2) Determine the absorption spectra between 250 nm and 400 nm of the test solution and the standard solution, as obtained from the Absorbance Test, as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit maxima and minima at the same wavelengths.

(3) To 50 mg (potency) each of Methacycline Hydrochloride and Methacycline Hydrochloride RS, add 80 mL of methanol, dissolve by shaking, add methanol to make 100 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ammonia water and methanol (1.5:100) to a distance of about 10 cm. Examine under ultraviolet light (main wavelength: 254 nm) or spray 0.1 mol/L potassium permanganate VS:

the spots obtained from the test solution and the standard solution show the same R_f value.

Absorbance Weigh accurately about 50 mg (potency) each of Methacycline Hydrochloride and Methacycline Hydrochloride RS and dissolve in 0.01 mol/L hydrochloric acid-methanol TS to make exactly 100 mL. Pipet 10 mL each of these solutions, add 0.01 mol/L hydrochloric acid-methanol TS to make exactly 250 mL and use these solutions as the test solution and the standard solution. Determine the absorbances, A_T and A_S (88.4 ~ 96.4 %), at 345 nm of the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry, using 0.01 mol/L hydrochloric acid-methanol TS as the blank.

$$\text{Absorbance (\%)} = \frac{A_T}{A_S} \times \frac{\text{Amount [}\mu\text{g (potency)] of Methacycli ne Hydrochlor ide RS taken}}{\text{Amount (mg) of Methacycli ne Hydrochlor ide taken} \times 10}$$

pH The pH of a solution obtained by dissolving 1.0 g (potency) of Methacycline Hydrochloride in 100 mL of water is between 2.0 and 3.0.

Water Not more than 2.0 % (0.5 g, volumetric titration, direct titration).

Assay ***The Cylinder-plate method*** (1) Agar media for seed and base layer- Use the medium in I 2 1) (2) ② under Microbial Assay for Antibiotics.

(2) Test organism- *Micrococcus luteus* ATCC 9341

(3) Weigh accurately about 25 mg (potency) of Methacycline Hydrochloride, dissolve in 2.0 mL of methanol and add 0.01 mol/L hydrochloric acid TS so that each mL contains 1 mg (potency). Pipet a suitable amount of this solution, dilute with 0.01 mol/L phosphate buffer (pH 4.5) so that each mL contains 40.0 μg (potency) and 10.0 μg (potency) and use these solutions as the high concentration test solution and the low concentration test solution, respectively. Separately, weigh accurately about 25 mg (potency) of Methacycline Hydrochloride RS, dissolve in 2.0 mL of methanol, add 0.01 mol/L hydrochloric acid so that each mL contains 1 mg (potency) and use this solution as the standard stock solution. Keep this standard stock solution at not exceeding 5 °C and use within 7 days. Pipet a suitable amount of this standard stock solution, dilute with 0.1 mol/L phosphate buffer (pH 4.5) so that each mL contains 40.0 μg (potency) and 10.0 μg (potency) and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively. Perform the test with these solutions as directed in I 8 under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Methacycline Hydrochloride Capsules

Methacycline Hydrochloride Capsules contain not less than 90.0 % and not more than 120.0 % of the labeled amount of methacycline ($C_{22}H_{22}N_2O_8$: 442.42).

Method of Preparation Prepare as directed under Capsules, with Methacycline Hydrochloride.

Identification Weigh an amount of Methacycline Hydrochloride Capsules, equivalent to 50 mg (potency) of methacycline hydrochloride according to the labeled amount, and about 50 mg (potency) of Methacycline Hydrochloride RS and perform the test as directed in Identification (2) and (3) under Methacycline Hydrochloride.

Loss on Drying Not more than 5.0 % (0.1 g, 0.7 kPa, 60 °C, 3 hours).

Dissolution Test Perform the test with 1 capsule of Methacycline Hydrochloride Capsules at 100 revolutions per minute according to Method 1, using 900 mL of water as the dissolution solution. Take the dissolved solution after 60 minutes from the start of the test and filter. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL and use this solution as the test solution. Separately, weigh accurately a suitable amount of Methacycline Hydrochloride RS, dissolve in the dissolution solution to make the same concentration as the test solution and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 345 nm of the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry, using the dissolution solution as the blank. The dissolution rate of Methacycline Hydrochloride Capsules in 60 minutes is not less than 70 % (Q).

$$\text{Dissolution rate (\%) with respect to the labeled amount methacycline hydrochloride } (C_{22}H_{22}N_2O_8 \cdot HCl) = C_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 90000$$

C_S: Concentration [mg (potency)/mL] of the standard solution

C: Labeled amount [mg (potency)] of methacycline hydrochloride ($C_{22}H_{22}N_2O_8 \cdot HCl$) in 1 capsule

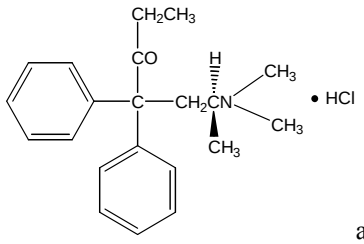
Uniformity of Dosage Units It meets the requirement.

Assay ***The Cyliner-plate method*** Perform the test as directed in the Assay under Methacycline Hydrochloride. Weigh accurately the mass of the contents of not less than 20 Methacycline Hydrochloride Capsules. Weigh accurately a portion of the contents, equivalent

to about 0.2 g (potency) according to the labeled potency, add diluted methanol (1 in 2) and shake well to make a solution containing 2 mg (potency) per mL. Filter, if necessary. Pipet a suitable amount of this solution, dilute with 0.1 mol/L phosphate buffer (pH 4.5) to make the concentration of (3) and use this solution as the test solution.

Containers and Storage ***Containers***—Tight containers.

Methadone Hydrochloride



and enantiomer

$C_{21}H_{27}NO \cdot HCl$: 345.91

6-(Dimethylamino)-4,4-diphenyl-3-heptanone hydrochloride [*1095-90-5*]

Methadone Hydrochloride contains not less than 98.5 % and not more than 100.5 % of methadone hydrochloride ($C_{21}H_{27}NO \cdot HCl$), calculated on the dried basis.

Description Methadone Hydrochloride is a colorless, white crystalline powder and odorless.
Methadone Hydrochloride is freely soluble in ethanol or in chloroform, soluble in water and practically insoluble in ether or in glycerin.

Identification (1) Determine the infrared spectra of Methadone Hydrochloride and Methadone Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) A solution of Methadone Hydrochloride responds to the Qualitative Test for chloride.

pH The pH of a solution of Methadone Hydrochloride (1 in 10) is between 4.5 and 6.5.

Purity ***Related substances***—Weigh 0.1 g of Methadone Hydrochloride, dissolve in 10 mL of ethanol and use this solution as the test solution. Separately, weigh 10 mg of Methadone Hydrochloride RS, previously dried at 105 °C for 1 hour and dissolve in 10 mL of ethanol. To 0.1 mL, 0.5 mL, 1.0 mL and 2.0 mL volumes of the test solution, add ethanol to make 10 mL and use this solutions as the standard solutions (1), (2), (3) and (4), respectively. Perform the test with the test solution and the standard solutions (1), (2), (3) and (4), as directed under the Thin-layer Chromatography. Spot 20 μL each of the test solution and standard solutions (1), (2), (3) and (4), on the plate. Develop the plate with a mixture of methanol and ammonia TS (200 : 3) to a distance of 15 cm and air-dry the plate. Spray the detecting agent on the plate. Intensities of spots other than the principal spot are not more than 1.0 %, relative to the principal spot.

Detecting agent—Dissolve 0.85 g of bismuth subnitrate in 40 mL of water and 10 mL of acetic acid (100) and use this solution as solution A. Dissolve 8 g of potassium iodide in 20 mL of water and use this solution as solution B. To 10 mL of the mixture of solution A and solution B, add 20 mL of acetic acid (100) to make 100 mL.

Loss on Drying Not more than 0.3 % (0.5 g, dried at 105 °C for 1 hour).

Residue on Ignition Not more than 0.1 % (1 g).

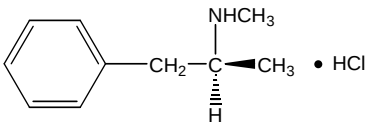
Assay Weigh accurately about 0.5 g of Methadone Hydrochloride, dissolve in a mixture of 10 mL of acetic acid (100) and 10 mL of mercuric acetate and warm slightly, if necessary. Cool the solution to room temperature, add 10 mL of dioxane and titrate rapidly with 0.1 mol/L perchloric acid VS (indicator: crystal violet TS) as directed under the Endpoint Detection Method in Titrimetry. Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid
= 34.591 mg of $C_{21}H_{27}NO \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Methamphetamine Hydrochloride



$C_{10}H_{15}N \cdot HCl$: 185.69

(*S*)-*N*-Methyl-1-phenylpropan-2-amine hydrochloride [*51-57-0*]

Methamphetamine Hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of methamphetamine hydrochloride ($C_{10}H_{15}N \cdot HCl$).

Description Methamphetamine Hydrochloride ap-

pears as colorless crystals or white, crystalline powder and is odorless.
Methamphetamine Hydrochloride is freely soluble in water, in ethanol or in chloroform and practically insoluble in ether.

pH—A solution of Methamphetamine Hydrochloride (1 in 10) is between 5.0 and 6.0.

Identification (1) Take 5 mL of a solution of Methamphetamine Hydrochloride (1 in 100) and add 0.5 mL of chloroplatinic acid TS: an orange-yellow, crystalline precipitate is produced.

(2) Take 5 mL of a solution of Methamphetamine Hydrochloride (1 in 100) and add 0.5 mL of iodine TS: a brown precipitate is produced.

(3) Take 5 mL of a solution of Methamphetamine Hydrochloride (1 in 100) and add 0.5 mL of picric acid TS: a yellow, crystalline precipitate is produced.

(4) A solution of Methamphetamine Hydrochloride (1 in 20) responds to the Qualitative Tests for chloride.

Specific Optcal Rotation $[\alpha]_D^{20}$: +16 ~ +19° (0.2 g, after drying, water, 10 mL, 100 mm).

Melting Point 171 ~ 175 °C.

Purity (1) ***Acid or alkali***—Dissolve 2.0 g of Methamphetamine Hydrochloride in 40 mL of freshly boiled and cooled water, add 2 drops of methyl red TS and use this solution as the test solution. (i) To 20 mL of the test solution, add 0.20 mL of 0.01 mol/L sulfuric acid VS: a red color is observed. (ii) To 20 mL of the test solution, add 0.20 mL of 0.02 mol/L sodium hydroxide VS: a yellow color is observed.

(2) ***Sulfate***—Dissolve 50 mg of Methamphetamine Hydrochloride in 40 mL of water, add 1 mL of dilute hydrochloric acid and 1 mL of barium chloride TS and allow to stand for 10 minutes: the solution remains unchanged.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

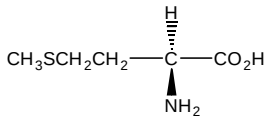
Assay Weigh accurately about 0.4 g of Methamphetamine Hydrochloride, previously dried and dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3). Titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 18.569 mg of $C_{10}H_{15}N \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

DL-Methionine



and enantiomer

$C_5H_{11}NO_2S$:149.21

(*RS*)-2-Amino-4-methylsulfanylbutanoic acid [*59-51-8*]

DL-Methionine contains not less than 99.0 % and not than 101.0 % of DL-methionine ($C_5H_{11}NO_2S$), calculated on the dried basis.

Description DL-Methionine appears as white crystals or crystalline powder.
DL-Methionine is sparingly soluble in water, slightly soluble in ethanol (95) and practically insoluble in ether.
DL-Methionine dissolves in dilute hydrochloric acid or in dilute sodium hydroxide TS.

Melting point—About 270 °C.

Identification (1) Dissolve about 0.1 g of DL-Methionine and 0.1 g of glycine in 4.5 mL of 2 mol/L sodium hydroxide TS, add 1 mL of 2.5 % sodium pentacyanonitrosylferrate (III) dihydrate solution and heat at 40 °C for 10 minutes. After cooling, add 2 mL of a mixture of hydrochloric acid and phosphoric acid (9:1): the color changes to deep red.

(2) Determine the infrared spectra of DL-Methionine and DL-Methionine RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) When proceed as directed in the Related substances under the Purity, the R_f value and color of the principal spot from the test solution (2) are same as those from the standard solution (1).

Specific Optical Rotation $[\alpha]_D^{20}$: -0.05 ~ +0.05° (2.5 g, 1 mol/L hydrochloric acid TS, 50 mL, 100 mm).

pH Dissolve 1.0 g of DL-Methionine in 50 mL of water: the pH of this solution is between 5.4 and 6.1.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of DL-Methionine in 50 mL of water: the solution is clear and colorless.

(2) ***Chloride***—Dissolve 0.5 g of DL-Methionine in 20 mL of water and add 6 mL of dilute nitric acid and water to make 40 mL. Perform the test. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS, 6 mL of dilute nitric acid and water to make 40 mL. To the test solution and the control solution, add 10 mL each of silver nitrate TS (not more

than 0.021 %).

(3) ***Sulfate***—Proceed with 1.0 g of DL-Methionine in 20 mL of water, heat at 60 °C, then cool at 10 °C, add water to make 50 mL. Prepare the control solution with 0.4 mL of 0.005 mol/L sulfuric acid VS (not more than 0.02 %).

(4) ***Heavy metals***—Proceed with 1.0 g of DL-Methionine according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(5) ***Related substances***—Dissolve 0.2 g of DL-Methionine in water to make exactly 10 mL and use this solution as the test solution (1). Pipet 1.0 mL of the test solution and add water to make exactly 50 mL and use this solution as the test solution (2). Separately dissolve 20 mg of DL-Methionine in water to make exactly 50 mL and use this solution as the standard solution (1). Pipet 1.0 mL of this solution, add water to make exactly 10 mL and use this solution as the standard solution (2). Perform the test with the test solutions and the standard solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution (1), test solution (2), standard solution (1) and standard solution (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (60 : 20 : 20) to a distance of about 10 cm and air-dry the plate. Spray evenly ninhydrin TS for spraying on the plate and heat the plate at 100 to 105 °C for 15 minutes: the spots other than the principal spot from the test solution (1) are not more intense than the spot from the standard solution (2) (not more than 0.2 %).

Loss on Drying Not more than 0.5 % (1 g, 105 °C).

Residue on Ignition Not more than 0.1 % (1 g).

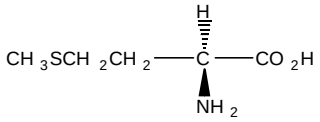
Assay Weigh accurately about 0.14 g of DL-Methionine, dissolve in 3 mL of anhydrous formic acid, add 30 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 14.921 mg of $C_5H_{11}NO_2S$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

L-Methionine



$C_5H_{11}NO_2S$: 149.21

(2*S*)-2-Amino-4-methylsulfanylbutanoic acid [*63-68-3*]

L-Methionine, when dried, contains not less than 98.5 % and not more than 101.0 % of L-methionine ($C_5H_{11}NO_2S$).

Description L-Methionine appears as white crystals or crystalline powder and has a characteristic odor.
L-Methionine is freely soluble in formic acid, soluble in water, very slightly soluble in ethanol (95).
L-Methionine dissolves in dilute hydrochloric acid.

Identification (1) Add 25 mg of L-Methionine to 1 mL of sulfuric acid saturated with anhydrous cupric sulfate: a yellow color is observed.

(2) Add 1 mL of ninhydrin TS to 5 mL of L-Methionine solution (1 in 5000) and heat on a water-bath for 2 minutes: a blue-purple color is observed.

(3) Dissolve 0.3 g of L-Methionine in 10 mL of water and add 10 drops of dilute hydrochloric acid and 2 mL of sodium nitrate TS: a colorless gas is evolved with effervescence.

pH Dissolve 0.5 g of L-Methionine in 20 mL water: the pH of this solution is between 5.2 and 6.2.

Specific Optical Rotation $[\alpha]_D^{20}$: +21.0 ~ +25.0° (0.5 g, after drying, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of L-Methionine in 20 mL of water: the solution is clear and colorless.

(2) ***Chloride***—Dissolve 0.5 g of in L-Methionine 20 mL of water and add 6 mL of dilute nitric acid and water to make 40 mL. Perform the test. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS, 6 mL of dilute nitric acid and water to make 40 mL. To the test solution and the control solution, add 10 mL each of silver nitrate TS (not more than 0.021 %).

(3) ***Sulfate***—Perform the test with 0.5 g of L-Methionine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048 %).

(4) ***Ammonium***—Perform the test with 0.25 g of L-Methionine. Prepare the control solution with 5.0 mL of standard ammonium solution (not more than 0.028 %).

(5) ***Heavy metals***—Dissolve 1.0 g of L-Methionine in 40 mL of water and 2 mL of dilute acetic acid, dissolve by warming, cool and add water to make 50 mL. Perform the test. Prepare the control solution as follows: to 2.0 mL of standard lead solution, add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(6) ***Iron***—Dissolve about 0.333 g of L-Methionine in water to make 45 mL, add 2 mL of hydrochloric acid

and use this solution as the test solution. To 1.0 mL of iron standard solution, add water to make 45 mL, add 2 mL of hydrochloric acid and use this solution as the standard solution. To each of the test solution and the standard solution, add 50 mg of ammonium peroxydisulfate and 3 mL of 30 % ammonium thiocyanate solution: the test solution has no more color than the standard solution (not more than 30 ppm).

(7) ***Arsenic***—Transfer 1.0 g of L-Methionine to a decomposition flask, add 5 mL of nitric acid and 2 mL of sulfuric acid, put a small funnel on the mouth of the flask and heat carefully until white fumes are evolved. After cooling, add two 2 mL volumes of nitric acid, heat, add 2 mL volumes of hydrogen peroxide (30) several times and heat again until the solution becomes colorless to pale yellow. After cooling, add 2 mL of saturated ammonium oxalate monohydrate solution and heat again until white fumes are evolved. After cooling, add water to make 5 mL and perform the test with this solution as the test solution (not more than 2 ppm).

(8) ***Related substances***—Dissolve 0.10 g of L-Methionine in 10 mL of water and use this solution as the test solution. Pipet 1.0 mL of the test solution and add water to make exactly 50 mL. Pipet 5.0 mL of this solution, add water to make exactly 20 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. After air-drying, develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3 : 1 : 1) to a distance of about 10 cm and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate and heat at 80 °C for 5 minutes: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.3 % (1 g, 105 °C, 4 hours).

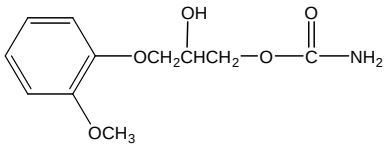
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.15 g of L-Methionine, previously dried and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 14.921 mg of $C_5H_{11}NO_2S$

Containers and Storage ***Containers***—Tight containers.

Methocarbamol



and enantiomer

$C_{11}H_{15}NO_5$: 241.24

2-Hydroxy-3-(2-methoxyphenoxy)propyl carbamate [*532-03-6*]

Methocarbamol contains not less than 98.5 % and not more than 101.5 % of methocarbamol ($C_{11}H_{15}NO_5$), calculated on the dried basis.

Description Methocarbamol appears as white powder and is odorless or has a characteristic odor.
Methocarbamol is soluble in heated ethanol, sparingly soluble in chloroform and practically insoluble in benzene or in n-hexane.

Identification (1) Determine the absorption spectra of Methocarbamol and Methocarbamol RS in ethanol (95) solution (1 in 25000) as directed under Ultraviolet-visible Spectrophotometery: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Methocarbamol and Methocarbamol RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity (1) ***Heavy metals***—Dissolve 1.0 g of Methocarbamol in 7 mL of methanol and 3 mL of 1 mol/L acetic acid, add water to make 25 mL and perform the test according to Method 1. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Dissolve 0.1 g of Methocarbamol in 13 mL of methanol, add pH 4.5 buffer solution to make 50 mL and use this solution as the test solution. Use this solution within 24 hours. Dissolve 20.0 mg of Guanefesin RS in methanol to make exactly 50 mL. Separately, weigh 20 mg of Methocarbamol RS in 1.0 mL of this solution and 2.0 mL of methanol and add pH 4.5 buffer solution to make exactly 10 mL. Use this solution as the standard solution. Use this solution within 24 hour after preparation. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Measure the peak area of methocarbamol and the peak areas of all peaks of which relative retention times to methocarbamol are greater 0.5 (not more than 2.0 %).

$$\text{Amount (\%) of related substances} = 100 \times \frac{2.4}{A_S} \times \frac{A_i}{A_T}$$

A_S: Peak area (%) of Guanefesin in the standard solution

A_i: Peak area of all related substances in the test solution

A_T: Peak areas of all related substances and methocarbamol in the test solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm to 10 μm in particle diameter).

Mobile phase: A mixture of pH 4.5 buffer solution and methanol (75 : 25).

System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the percentage of the peak area of guanefesin is 2.4 ± 1.0 % and guanefesin and methocarbamol are eluted in this order with the resolution between their peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area percentage is not more than 4.0 %.

pH 4.5 buffer solution—Dissolve 6.8 g of monobasic potassium phosphate to 1000 mL of water and titrate with 6 mol/L of phosphoric acid or 10 mol/L of potassium hydroxide to the pH of 4.5 ± 0.05.

Loss on Drying Not more than 0.5 % (1 g, 60 °C, 2 hours).

Loss on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.1 g of Methocarbamol, transfer to a volumetric flask and add methanol to make 100 mL. Pipet 4.0 mL of this solution, transfer to a volumetric flask, add methanol to make 100 mL and use this solution as the test solution. Separately, weigh accurately about 0.1 g of Methocarbamol RS, dissolve in methanol, proceed as directed with the test solution and use this solution as the standard solution. Determine the absorbances, A_T and A_S, as directed under Ultraviolet-visible spectrophotometry at the wavelength of maximum absorbance at about 274 nm for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of methocarbamol } (C_{11}H_{15}NO_5) = \text{Amount (mg) of Methocarbamol RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Tight containers.

Methocarbamol Injection

Methocarbamol Injection is an aqueous solution for injection. Methocarbamol Injection contains not less than 95.0 % and not more than 105.0 % of the labeled amount of methocarbamol ($C_{11}H_{15}NO_5$: 241.24).

Method of Preparation Prepare as directed under Injections, with Methocarbamol.

Description Methocarbamol Injection is a clear, colorless liquid.

Identification Mix a volume of Methocarbamol Injection, equivalent to about 500 mg of Methocarbamol, with 40 mL of water in a small separator. Extract with 10 mL of ethyl acetate and dry the ethyl acetate layer over sodium sulfate anhydrous. Evaporate the ethyl acetate in a 40 °C water-bath with nitrogen gas and the Methocarbamol so obtained responds to the Identification (1) under Methocarbamol.

pH 3.5 ~ 6.0.

Purity ***Aldehydes***—Transfer an accurately measured volume of Methocarbamol Injection to a volumetric flask, equivalent to 400 mg of Methocarbamol, add 2.0 mL of filtered solution of phenylhydrazine hydrochloride (1 in 100) in diluted ethanol (1 in 5) and allow to stand for 10 minutes. Add 1 mL of potassium hexacyanoferrate (III) solution (1 in 100) and allow to stand for 5 minutes. Add 4 mL of hydrochloric acid, dilute with ethanol (95) to make exactly 25 mL and use this solution as the test solution. Separately pipet 4.0 mL of formaldehyde solution (1 in 100000) and prepare under the same conditions as the test solution and use this solution as the standard solution. Determine the absorption spectra of the test solution and the standard solution as directed under Ultraviolet-visible spectrophotometry: the absorbance of the test solution exhibit not more than 0.01 % of the absorbance of the standard solution at the wavelength of maximum absorption at about 515 nm.

Sterility Test It meets the requirement.

Bacterial Endotoxins Methocarbamol Injection contains less than 0.2 EU/mg of Methocarbamol.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Container It meets the requirement.

Assay Accurately transfer a volume of Methocarbamol Injection, equivalent to 0.1 g of Methocarbamol, to a flask. Add mobile phase to make exactly 100 mL and use this solution as the test solution. Separately weigh accurately about 0.1 g of Methocarbamol RS to a flask, dissolve in the mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography. Measure the peak areas, A_T and A_S, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of methocarbamol } (C_{11}H_{15}NO_5) = \text{Amount (mg) of Methocarbamol RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm to 5 μm in particle diameter).

Column temperature: A constant temperature of about 30 °C.

Mobile phase: A mixture of pH 4.5 buffer solution and methanol (70 : 30).

Flow rate: 1 mL/minute.

System suitability

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methocarbamol is not more than 2.0 %.

pH 4.5 buffer solution—Dissolve 6.8 g of monobasic potassium phosphate in 1000 mL of water and adjust the pH to 4.5 ± 0.05 with 6 mol/L phosphoric acid TS or 10 mol/L potassium hydroxide TS.

Containers and Storage ***Containers***—Hermetic containers.

Methocarbamol Tablets

Methocarbamol Tablets contain not less than 95.0 % and not more than 105.0 % of the labeled amount of methocarbamol ($C_{11}H_{15}NO_5$: 241.24).

Method of Preparation Prepare as directed under Tablets, with Methocarbamol.

Identification Mix a volume of finely powdered Tablet, equivalent to 1 g of Methocarbamol, with 25 mL of water in a separatory funnel and extract with 25 mL of chloroform. Filter the extract and evaporate to dryness: the residue so obtained responds to the Identification (1) under Methocarbamol.

Dissolution Test Perform the test with 1 tablet of Methocarbamol Tablets at 50 revolutions per minute according to the Method 2 under the Dissolution Test, using 900 mL of the water as the dissolution solution. After 45 minutes from the start of the test, proceed with the dissolved solution as directed in the Assay.
The dissolution rate of Methocarbamol Tablets in 45 minutes is not less than 75 %.

Uniformity of Dosage Units It meets the requirement.

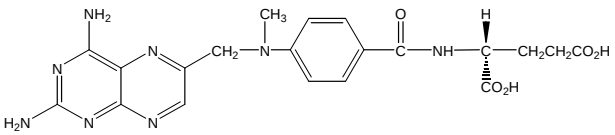
Assay Weigh accurately and powder not less than 20 Methocarbamol Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of methocarbamol ($C_{11}H_{15}NO_5$) to a flask. Add about 50 mL of pH 4.5 buffer solution, 25 mL of methanol and 5.0 mL of the internal standard solution. Shake vigorously for 10 minutes, dilute with pH 4.5 buffer solution to make exactly 100 mL. Filter this solution, discard the first 20 mL of the filtrate and use the subsequent filtrate as the tst solution. Separately, weigh accurately about 0.1 g of Methocarbamol RS, dissolve in about 50 mL of pH 4.5 buffer solution and 25 mL of methanol, add 5.0 mL of the internal standard solution, add pH 4.5 buffer solution to make exactly 100 mL and use this solution as the standard solution. Proceed as directed in the Assay under Methocarbamol Injection.

$$\text{Amount (mg) of methocarbamol } (C_{11}H_{15}NO_5) = \text{Amount (mg) of Methocarbamol RS} \times \frac{A_T}{A_S}$$

Internal standard solution—A 3 mg/mL solution of caffeine in methanol

Containers and Storage ***Containers***—Tight containers.

Methotrexate



$C_{20}H_{22}N_8O_5$: 454.44

(2*S*)-2-[[4-[(2,4-Diaminopteridin-6-yl)methyl-methylamino]benzoyl]amino]pentanedioic acid
[*59-05-2*]

Methotrexate is a mixture of 4-amino-10-methylfolic acid and closely related compounds. Methotrexate contains not less than 94.0 % and not more than 102.0 % of methotrexate ($C_{20}H_{22}N_8O_5$), calculated on the anhydrous basis.

Description Methotrexate appears as yellow-brown, crystalline powder.
Methotrexate is slightly soluble in pyridine and practically insoluble in water, in ethanol (95), in acetonitrile or in ether.
Methotrexate dissolves in dilute sodium hydroxide TS or in dilute sodium carbonate TS.
Methotrexate is gradually colored by light.

Identification (1) Determine the absorption spectra of the solutions of 1 mg each of Methotrexate and Methotrexate RS in 100 mL of 0.1 mol/L hydrochloric acid TS as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Methotrexate and Methotrexate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity (1) ***Heavy metals***—Weigh 1.0 g of Methotrexate, add 4 mL of a solution of magnesium sulfate heptahydrate in dilute sulfuric acid (1 in 4), mix, heat in a water-bath and evaporate to dryness. Ignite the residue to incinerate at not exceeding 800 °C. Allow to cool and moisten the residue with a small amount of dilute sulfuric acid. Evaporate to dryness and ignite to incinerate within 2 hours. Allow to cool, take two portions of the residue and to each, 5 mL of 2 mol/L hydrochloric acid TS. Add 0.1 mL of phenolphthalein TS and add ammonia solution (28) dropwise until the solution develops a pale red color. Allow to cool, add acetic acid (100) until the color disappears and add a further 0.5 mL. Filter and wash, if necessary. Add water to make 20 mL and use this solution as the test solution. Separately, prepare a solution in the same manner as the test solution, using 5.0 mL of standard lead solution instead of Methotrexate. To 10 mL of this solution, add 2 mL of the test solution and use this solution as the control solution. Separately, add 2 mL of the test solution to 10 mL of water and use this solution as the blank solution. To 12 mL each of the test solution, the control solution and the blank solution, add 2 mL of pH 3.5 acetate buffer, mix, add 1.2 mL of thioacetamide TS and mix immediately. Allow to stand for 2 minutes: the test solution has no more color than the control solution (not more than 50 ppm).

System suitability: The control solutions shows a faint brown color compared to the blank solution. To the test solution, add 5.0 mL of standard lead solution. To 10 mL of this solution, add 2 mL of the test solution and use this solution as the system suitability solution. The color of the system suitability solution is the same as or more intense than that of the control solution.

(2) ***Related substances***—Weigh accurately about 100 mg of Methotrexate, dissolve in the mobile phase to make exactly 100 mL and use this solution as the test solution. Separately, weigh accurately an amount each of Methotrexate RS, methotrexate related substance I {[(S)-2-{4-[(2,4-diaminopteridin-6-yl)methylamino]benzamido}
pentanedioic acid} RS, methotrexate related substance II {(S)-2-(4-{[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl](methyl)amino}benzamido)pentanedioic
acid} RS and methotrexate related substance III {4-{[(2,4-diaminopteridin-6-
yl)methyl]methylamino}benzoic acid 1/2 hydrochloride} RS, dissolve in the mobile phase to make a solution containing 0.003 mg each per mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the amount of each related substances according to equation (1): the amount of methotrexate related substance I is not more than 0.3 % and the amount of methrotrexate related substance II is not more than 0.5 %. Determine the amount of 4-[[(2,4-diaminopteridin-6-yl)methyl]methyl-amino]benzoic
acid according to equation (2): not more than 0.3 %. Determine the amount of each related substance according to equation (3): not more than 0.1 %, and the total amount of related substances is not more than 0.5 %. Disregard any peak having an area smaller than 0.1 times the peak area of methotrexate from the standard solution.

$$\text{Amount (\%) of related substances} = 100 \times \frac{C_S}{C_T} \times \frac{A_T}{A_S} \qquad (1)$$

C_S: Concentration (mg/mL) of each related substance in the standard solution

C_T: Concentration (mg/mL) of methotrexate in the test solution

A_T: Peak area of each related substance from the test solution

A_S: Peak area of each related substance from the standard solution

$$\text{Amount (\%) of 4-[[(2,4-diaminopteridin-6-yl)methyl]methylamino]benzoic acid} = 100 \times \frac{325.33}{343.56} \times \frac{C_S}{C_T} \times \frac{A_T}{A_S} \qquad (2)$$

325.33: Molecular mass of 4-[[(2,4-diaminopteridin-6-yl)methyl]methylamino]benzoic acid

343.56: Molecular mass of methotrexate related substance III {4-{[(2,4-diaminopteridin-6-yl)methyl]methyl-amino}benzoic acid 1/2 hydrochloride}

C_S: Concentration (mg/mL) of methotrexate related substance III in the standard solution

C_T: Concentration (mg/mL) of methotrexate in the test solution

A_T: Peak area of 4-[[(2,4-diaminopteridin-6-yl)methyl]
methylamino]benzoic acid from the test solution

A_S: Peak area of 4-[[(2,4-diaminopteridin-6-yl)methyl]
methylamino]benzoic acid from the standard solution

$$\text{Amount (\%) of each related substance} = 100 \times \frac{C_S}{C_T} \times \frac{A_T}{A_S} \qquad (3)$$

C_S: Concentration (mg/mL) of methotrexate in the standard solution

C_T: Concentration (mg/mL) of methotrexate in the test solution

A_i: Peak area of each related substance from the test solution

A_S: Peak area of methotrexate from the standard solution

Operating conditions

Detector and column: Proceed as directed in the operating conditions in the Assay.

Mobile phase: To 900 mL of pH 6.0 disodium hydrogen phosphate-citric acid buffer, add 100 mL of acetonitrile and mix.

Flow rate: 1.2 mL/minute

Relative retention time: The relative retention time of methotrexate related substance I, methotrexate related substance II and methotrexate related substance III with respect to methotrexate is about 0.59, 0.52 and 2.16, respectively.

System suitability

System performance: Dissolve 10 mg each of Methotrexate and folic acid in 100 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, the relative retention time of folic acid with respect to the retention time of methotrexate is 0.35 with the resolution between their peaks being not less than 8.0.

Time span of measurement: About 3 times as long as the retention time of methotrexate.

Water Take 5 mL of pyridine for water determination and 20 mL of methanol for water determination in a dried titration flask and titrate with Karl Fischer TS until the end point is achieved. Weigh accurately about 0.2 g of Methotrexate, immediately place in the titration flask and add a known excess volume of Karl Fischer TS. Mix well for 30 minutes and perform the test: the water content is not more than 12.0 %.

Residue on Ignition Not more than 0.1 % (0.5 g).

Assay Weigh accurately about 25 mg each of Methotrexate and Methotrexate RS, dissolve each in the mobile phase to make exactly 250 mL and use these as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according the following operating conditions and measure the peak areas, A_T and A_S, of Methotrexate for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of methotrexate } (C_{20}H_{22}N_8O_5) = \text{Amount (mg) of Methotrexate RS, calculated on the anhydrous basis} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 302 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Mobile phase: A mixture of 890 mL of dibasic sodium phosphate-citric acid buffer solution, pH 6.0 and 110 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of methotrexate is about 8 minutes.

System suitability

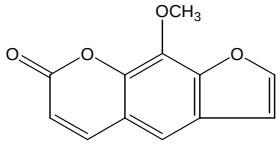
System performance: Dissolve 10 mg each of Methotrexate and folic acid in 100 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, folic acid and methotrexate are eluted in this order with the resolution between their peaks being not less than 8.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of methotrexate is not more than 2.5 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Methoxsalen



$C_{12}H_8O_4$: 216.19

9-Methoxyfuro[3,2-g]chromen-7-one [*298-81-7*]

Methoxsalen contains not less than 98.0 % and not more than 102.0 % of methoxsalen ($C_{12}H_8O_4$), calculated on the anhydrous basis.

Description Methoxsalen appears as white to pale yellow crystals or crystalline powder, is odorless and tasteless.
Methoxsalen is freely soluble in chloroform, slightly soluble in methanol, in ethanol (95) or in ether and practically insoluble in water.

Identification (1) Take 10 mg of Methoxsalen, add 5 mL of dilute nitric acid and heat: a yellow color is observed. Make this solution alkaline with a solution of sodium hydroxide (2 in 5): the color changes to red-brown.

(2) Take 10 mg of Methoxsalen, add 5 mL of sulfuric acid and shake: a yellow color is observed.

(3) Determine the absorption spectra of the solutions of Methoxsalen and Methoxsalan RS in ethanol (95) (1 in 200000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting Point 145 ~ 149 °C.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Methoxsalen according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Arsenic***—Prepare the test solution with 1.0 g of Methoxsalen according to Method 3 and perform the test (not more than 2 ppm).

(3) ***Related substances***—Dissolve 50 mg of Methoxsalen in 10 mL of chloroform and use this solution as the test solution. Pipet 2.0 mL of the test solution and add chloroform to make exactly 50 mL. Pipet 1.0 mL of this solution, add chloroform to make exactly 10 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 mL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, hexane and ethyl acetate (40 : 10 : 3) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than spot from the standard solution.

Water Not more than 0.5 % (1 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

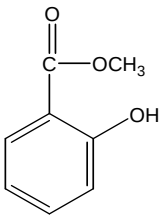
Assay Weigh accurately about 50 mg each of Methoxsalen and Methoxsalen RS and dissolve each in ethanol (95) to make exactly 100 mL. Pipet 2.0 mL each of these solutions and dilute each with ethanol (95) to make exactly 25 mL. Pipet 10.0 mL each of these solutions and dilute each again with ethanol (95) to make exactly 50 mL and use these solutions as the test solution and the standard solution, respectively. Determine the absorbances, A_T and A_S, of the test solution and the standard solution at 300 nm as directed under Ultraviolet-visible Spectrophotometry, respectively.

$$\text{Amount (mg) of methoxsalen } (C_{12}H_8O_4) = \text{Amount (mg) of Methoxsalen RS, calculated on the anhydrous basis} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Methyl Salicylate



$C_8H_8O_3$: 152.15

Methyl 2-hydroxybenzoate [*119-36-8*]

Methyl Salicylate contains not less than 98.0 % and not more than 101.9 % of methyl salicylate ($C_8H_8O_3$).

Description Methyl Salicylate is a colorless to pale yellow liquid and has a strong and characteristic odor.
Methyl Salicylate is miscible with ethanol (95) or ether.
Methyl Salicylate is very slightly soluble in water.

Specific gravity— d_{20}^{20}: 1.182 ~ 1.192.

Boiling point—219 ~ 224 °C.

Identification Shake 1 drop of Methyl Salicylate thoroughly with 5 mL of water for 1 minute and add 1 drop of iron (III) chloride TS: a purple color develops.

Purity (1) ***Acid***—Shake 5.0 mL of Methyl Salicylate thoroughly with 25 mL of freshly boiled and cooled water and 1.0 mL of 0.1 mol/L sodium hydroxide VS for 1 minute, add 2 drops of phenol red TS and titrate with 0.1 mol/L hydrochloric acid VS until the red color disappears: not more than 0.45 mL of 0.1 mol/L sodium hydroxide VS is consumed.

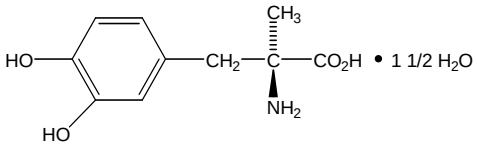
(2) ***Heavy metals***—Proceed with 1.0 g of Methyl Salicylate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

Assay Weigh accurately about 2 g of Methyl Salicylate, add an exactly measured 50 mL of 0.5 mol/L potassium hydroxide-ethanol VS and heat in a water-bath for 2 hours under a reflux condenser. Cool and titrate the excess potassium hydroxide with 0.5 mol/L hydrochloric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination and make any necessary correction.

Each mL of 0.5 mol/L potassium hydroxide-ethanol VS
= 76.07 mg of $C_8H_8O_3$

Containers and Storage ***Containers***—Tight containers.

Methyldopa Hydrate



Methyldopa $C_{10}H_{13}NO_4 \cdot 1^1/_2H_2O$: 238.24

(2*S*)-2-Amino-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid sesquihydrate [*41372-08-1*]

Methyldopa Hydrate contains not less than 98.0 % and not more than 101.0 % of methyldopa ($C_{10}H_{13}NO_4$: 211.22), calculated on the anhydrous basis.

Description Methyldopa Hydrate is a white to pale grayish white, crystalline powder.
Methyldopa Hydrate is slightly soluble in water, in methanol or in acetic acid (100), very slightly soluble in ethanol (95) and practically insoluble in ether.
Methyldopa Hydrate dissolves in dilute hydrochloric acid.

Identification (1) Take 10 mg of Methyldopa Hydrate, add 3 drops of ninhydrin TS and heat on a water-bath for 3 minutes: A purple color is observed.

(2) Determine the absorption spectra of solutions of Methyldopa Hydrate and Methyldopa Hydrate RS in 0.1 mol/L hydrochloric acid TS (1 in 25000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Methyldopa Hydrate and Methyldopa Hydrate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: -25 ~ -28° (1 g, calculated on the anhydrous basis, aluminum chloride TS, 20 mL, 100 mm).

Purity (1) ***Acid***—Shake 1.0 g of Methyldopa Hydrate with 100 mL of freshly boiled and cooled water and add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 2 drops of methyl red TS: a yellow color is observed.

(2) ***Chloride***—Perform the test with 0.5 g of Methyldopa Hydrate. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.028 %).

(3) ***Heavy metals***—Proceed with 2.0 g of Methyldopa Hydrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(4) ***Arsenic***—Prepare the test solution with 1.0 g of Methyldopa Hydrate in 5 mL of dilute hydrochloric acid and perform the test (not more than 2 ppm).

(5) ***3-O-Methylmethyldopa***—Dissolve 0.10 g of Methyldopa Hydrate in methanol to make exactly 10 mL and use this solution as the test solution. Separately, dissolve 5 mg of 3-*O*-methylmethyldopa RS in methanol to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 20 μL each of the test solution and the standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (13 : 5 : 3) to a distance of about 10 cm and air-dry the plate. Spray evenly 4-nitroaniline-sodium nitrite TS on the plate and air-dry the plate. Then spray evenly a solution of sodium carbonate (1 in 4) on the plate: the spot from the test solution corresponding to that from the standard solution is not more intense than the spot from the standard solution.

Water 10.0 ~ 13.0 % (0.2 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.3 g of Methyldopa Hydrate, dissolve in 80 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 2 to 3 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 21.121 mg of $C_{10}H_{13}NO_4$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Methyldopa Tablets

Methyldopa Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of methyldopa ($C_{10}H_{13}NO_4$: 211.21).

Method of Preparation Prepare as directed under Tablets, with Methyldopa Hydrate.

Identification (1) Take a portion of powdered Methyldopa Tablets, equivalent to 0.1 g of Methyldopa according to the labeled amount, add 10 mL of water and heat on a water-bath for 5 minutes with occasional shaking. After cooling, centrifuge for 5 minutes at 2000 rotations per minute, apply 1 drop of the clear supernatant liquid to a filter paper and dry with warm air. Place 1 drop of ninhydrin TS over the spot and heat for 5 minutes at 100 °C: a purple color is observed.

(2) Take 0.5 mL of the clear supernatant liquid obtained in the Identification (1), add 2 mL of 0.05 mol/L sulfuric acid TS, 2 mL of iron (II) tartrate TS and 4 drops of ammonia TS and shake well: a dark purple color is observed.

(3) Take 0.7 mL of the clear supernatant liquid obtained in the Identification (1) and add 0.1 mol/L hydrochloric acid TS to make 20 mL. To 10 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make 100 mL and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 277 nm and 283 nm.

Dissolution Test Perform the test with 1 tablet of Methyldopa Tablets at 50 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of water as the dissolution solution. Take 30 mL or more of the dissolved solution after 60 minutes from the start of the test and filter through a membrane filter with a pore size of not more than 0.8 μm. Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add water to make exactly V' mL so that each mL contains about 25 μg of methyldopa ($C_{10}H_{13}NO_4$: 211.22) according to the labeled amount and use this solution as the test solution. Separately, weigh accurately about 56 mg of Methyldopa RS (separately determine its loss on drying at 125 °C for 2 hours) and dissolve in water to make exactly 200 mL. Pipet 10.0 mL of this solution, add water to make exactly 100 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution at 280 nm as directed under Ultraviolet-visible Spectrophotometry, respectively.
The dissolution rate of Methyldopa Tablets in 60 minutes should be not less than 75 %.

Dissolution rate (%) with respect to the labeled amount of

$$\text{methyldopa } (C_{10}H_{13}NO_4) = W_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 45$$

W_S: Amount (mg) of Methyldopa RS, calculated on the dried basis

C: Labeled amount (mg) of methyldopa ($C_{10}H_{13}NO_4$: 211.22) in 1 tablet.

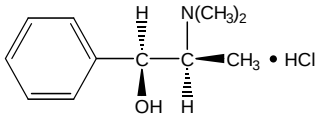
Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Methyldopa Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of methyldopa ($C_{10}H_{13}NO_4$), add 50 mL of 0.05 mol/L sulfuric acid TS, shake thoroughly for 15 minutes, add 0.05 mol/L sulfuric acid TS to make exactly 100 mL and filter through a dry filter paper. Discard the first 20 mL of the filtrate and use the subsequent filtrate as the test solution. Separately, weigh accurately about 0.11 g of Methyldopa RS (separately determine its loss on drying at 125 °C for 2 hours), dissolve in 0.05 mol/L sulfuric acid TS to make exactly 100 mL and use this solution as the standard solution. Pipet 5.0 mL each of the test solution and the standard solution, add exactly 5 mL of iron (II) tartrate TS and add ammonia-ammonium acetate buffer solution, pH 8.5, to make exactly 100 mL. Perform the test with the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry, using a solution prepared with 5 mL of 0.05 mol/L sulfuric acid TS in the same manner, as the blank. Determine the absorbances, A_T and A_S, of the subsequent solutions of the test solution and the standard solution at 520 nm, respectively.

Amount (mg) of methyldopa ($C_{10}H_{13}NO_4$: 211.22)
= Amount (mg) of Methyldopa RS,
calculated on the dried basis $\times \frac{A_T}{A_S}$

Containers and Storage ***Containers***—Well-closed containers.

dl-Methylephedrine Hydrochloride



and enantiomer

$C_{11}H_{17}NO \cdot HCl$: 215.72

(*RS*)-2-(Dimethylamino)-1-phenylpropan-1-ol hydrochloride [*18760-80-0*]

dl-Methylephedrine Hydrochloride, when dried, con-

tains not less than 99.0 % and not more than 101.0 % of *dl*-methylephedrine hydrochloride ($C_{11}H_{17}NO \cdot HCl$).

Description *dl*-Methylephedrine Hydrochloride appears as colorless crystals or a white, crystalline powder, is odorless and has a bitter taste.
dl-Methylephedrine Hydrochloride is freely soluble in water, soluble in ethanol (95), slightly soluble in acetic acid (100) and practically insoluble in acetic anhydride or in ether.
A solution of *dl*-Methylephedrine Hydrochloride (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectra of solutions of *dl*-Methylephedrine Hydrochloride and *dl*-Methylephedrine Hydrochloride RS (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of *dl*-Methyl ephedrine Hydrochloride and *dl*-Methylephedrine Hydrochloride RS, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of *dl*-Methylephedrine Hydrochloride (1 in 10) responds to the Qualitative Tests for chloride.

Melting Point 207 ~ 211 °C.

pH The pH of a solution obtained by dissolving 1.0 g of *dl*-Methylephedrine Hydrochloride in 20 mL of water is between 4.5 and 6.0.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of *dl*-Methylephedrine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of *dl*-Methylephedrine Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) ***Related substances***—Dissolve 50 mg of *dl*-Methylephedrine Hydrochloride in 20 mL of water, and use this solution as the test solution. Pipet 1 mL of the test solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following method, and determine each peak area by the automatic integration method: the total area of the peaks other than the peak of methylephedrine is not more than the peak area of methylephedrine from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 257 nm)

Column: A stainless steel column, 4.6 mm in internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: Dissolve 13.6 g of potassium dihydrogen phosphate and 3 g of sodium 1-heptane sulfonate in 1000 mL of water, and adjust the pH to 2.5 with phosphoric acid. To 900 mL of this solution, add 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of methylephedrine is about 10 minutes.

System suitability

Test for required detectability: To exactly 2 mL of the standard solution, add water to make exactly 20 mL. Confirm that the peak area of methylephedrine obtained from 20 μL of this solution is equivalent to 7 to 13 % of that of methylephedrine obtained from 20 μL of the standard solution.

System performance: Dissolve 50 mg of *dl*-Methylephedrine Hydrochloride and 0.4 mg of methyl parahydroxybenzoate in 50 mL of water. When the procedure is run with 20 μL of this solution under the above operating conditions, methylephedrine and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methylephedrine is not more than 2.0 %.

Time span of measurement: About 2 times as long as the retention time of methylephedrine beginning after the solvent peak.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

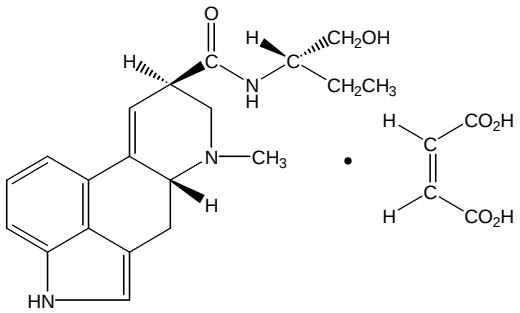
Assay Weigh accurately about 0.4 g of *dl*-Methylephedrine Hydrochloride, previously dried, dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 21.572 mg of $C_{11}H_{17}NO \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Methylergometrine Maleate



$C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$: 455.50

(6a*R*,9*R*)-*N*-[(2*S*)-1-Hydroxybutan-2-yl]-7-methyl-6,6a,8,9-tetrahydro-4*H*-indolo[4,3-fg]quinoline-9-carboxamide;(*Z*)-but-2-enedioic acid [*57432-61-8*]

Methylergometrine Maleate, when dried, contains not less than 95.0 % and not more than 105.0 % of methylergometrine maleate ($C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$).

Description Methylergometrine Maleate is a white to pale yellow, crystalline powder and is odorless.
Methylergometrine Maleate is slightly soluble in water, in methanol or in ethanol (95) and practically insoluble in ether.
The color of Methylergometrine Maleate is gradually changed to yellow by light.

Melting point—About 190 °C (with decomposition).

Identification (1) A solution of Methylergometrine Maleate (1 in 200) shows a blue fluorescence.

(2) The colored solution obtained in the Assay shows a deep blue color. Determine the absorption spectrum of the colored solution as directed under Ultraviolet-visible Spectrophotometry, and compare the spectrum with a solution of Methylergometrine Maleate RS prepared in the same manner as the test solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Take 5 mL of a solution of Methylergometrine Maleate (1 in 500) and add 1 drop of potassium permanganate TS: the red color of the solution fades immediately.

Specific Optical Rotation $[\alpha]_D^{20}$: +44 ~ +50° (0.1 g, after drying, water, 20 mL, 100 mm).

Purity ***Related substances***—Perform the test without exposure to daylight, using light-resistant vessels. Dissolve 8 mg of Methylergometrine Maleate in 2 mL of a mixture of ethanol (95) and ammonia solution (28) (9 : 1) and use this solution as the test solution. Pipet 1.0 mL of the test solution, add a mixture of ethanol (95) and ammonia solution (28) (9 : 1) to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography and immediately develop the plate with a mixture of chloroform, methanol and water (75 : 25 : 3) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 2.0 % (0.2 g, in vacuum, P_2O_5, 4 hours).

Assay Weigh accurately about 10 mg of Methylergometrine Maleate, previously dried, add water to make exactly 250 mL and use this solution as the test solution. Separately, weigh accurately about 10 mg of Methylergometrine Maleate RS, previously dried over silica gel for 4 hours, add water to make exactly 250 mL and use this solution as the standard solution. Pipet 2.0 mL each of the test solution and the standard solution into brown glass-stoppered test tubes, add 4.0 mL each of 4-dimethyl-aminobenzaldehyde-iron (III) chloride TS in ice-water and after heating for 10 minutes at 45 °C, allow to stand for 20 minutes at room temperature. Perform the test with the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry, using a solution, prepared with 2 mL of water in the same manner, as the blank. Determine the absorbances, A_T and A_S, of the subsequent solutions of the test solution and the standard solution at 545 nm, respectively.

$$\text{Amount (mg) of methylergometrine maleate } (C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4) = \text{Amount (mg) of Methylergometrine Maleate RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Methylergometrine Maleate Tablets

Methylergometrine Maleate Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of methylergometrine maleate ($C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$: 455.50).

Method of Preparation Prepare as directed under Tablets, with Methylergometrine Maleate.

Identification (1) The test solution obtained in the

Assay shows a blue fluorescence.

(2) The colored solution obtained in the Assay shows a deep blue color. Determine the absorption spectrum of the colored solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 543 nm and 547 nm and between 620 nm and 630 nm.

Dissolution Test Perform the test with 1 tablet of Methylergometrine Maleate Tablets at 100 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of water as the dissolution solution. Take 20 mL or more of the dissolved solution 30 minutes after starting the test and filter by membrane filter with a pore size of not exceeding 0.8 μm. Discard the first 10 mL of the filtrate and use the subsequent solution as the test solution, or pipet V mL of this filtrate, add water to make exactly V' mL so that each mL contains about 0.13 μg of methylergometrine maleate ($C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$) according to the labeled amount and use this solution as the test solution. Separately, weigh accurately about 25 mg of Methylergometrine Maleate RS, previously dried in a desiccator for 4 hours (in vacuum, P_2O_5) and dissolve in water to make exactly 100 mL. Pipet 5.0 mL of this solution, add water to make exactly 100 mL, then pipet 1.0 mL of this solution, add water to make exactly 100 mL and use this solution as the standard solution. Determine the fluorescence intensities, F_T and F_S, of the test solution and the standard solution, respectively, at 338 nm as the excitation wavelength and at 427 nm as the fluorescence wavelength as directed under the Fluorometry.

The dissolution rate of Methylergometrine Maleate Tablets in 30 minutes is not less than 70 %.

Dissolution rate (%) with respect to the labeled amount of methylergometrine maleate ($C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$) =

$$W_S \times \frac{F_T}{F_S} \times \frac{V'}{V} \times \frac{1}{C} \times 0.45$$

W_S: Amount (mg) of Methylergometrine Maleate RS.

C: Labeled amount (mg) of methylergometrine maleate ($C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$) in 1 tablet.

Uniformity of Dosage Units It meets the requirement when the content uniformity test is performed according to the following method.

Transfer 1 tablet of Methylergometrine Maleate Tablets to a brown glass-stoppered centrifuge tube, add 10 mL of water, shake for 10 minutes vigorously and disintegrate the tablet. Add 3 g of sodium chloride and 2 mL of ammonia solution (28), add exactly 25 mL of chloroform and after vigorous shaking for 10 minutes, centrifuge for 5 minutes. Discard the water layer, take the chloroform extracts, add chloroform to make exactly V mL of a solution containing about 5 μg of methylergometrine maleate ($C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$) per mL and use this solution as the test solution. Separately, weigh accurately about 1.3 mg of Methylergometrine Maleate RS, previously dried in a desiccator (silica gel) for 4 hours, dissolve in water and add water to make exactly 100 mL. Pipet 10.0 mL of this solution into a brown glass-stoppered centrifuge tube and add 3 g of sodium chloride and 2 mL of ammonia solution (28). Add exactly 25 mL of chloroform and after vigorous shaking for 10 minutes, centrifuge for 5 minutes. Discard the water layer, take the chloroform extract and use this solution as the standard solution. Pipet 20.0 mL each of the test solution and the standard solution into brown glass-stoppered centrifuge tubes, add immediately exactly 10 mL of dilute 4-dimethylaminobenzaldehyde-iron (III) chloride TS, respectively and shake for 5 minutes vigorously. Centrifuge the test solution and the standard solution for 5 minutes, take the water layers and allow to stand for 1 hour. Perform the test with the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry, using dilute 4-dimethylaminobenzaldehyde-iron (III) chloride TS, as the blank. Determine the absorbances, A_T and A_S, of the subsequent solutions of the test solution and the standard solution at 545 nm, respectively.

Amount (mg) of methylergometrine maleate ($C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$) = Amount (mg) of Methylergometrine Maleate RS $\times \frac{A_T}{A_S} \times \frac{V}{250}$

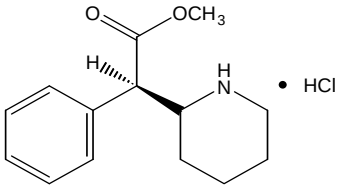
Assay Weigh accurately and powder not less than 20 Methylergometrine Maleate Tablets. Weigh accurately a portion of the powder, equivalent to about 0.3 mg of methylergometrine maleate ($C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$), transfer to a brown separatory funnel, add 15 mL of sodium bicarbonate solution (1 in 20) and extract with four 20 mL volumes of chloroform. Filter each volume of the chloroform extracts through a pledget of absorbent cotton, previously moistened with chloroform into another dried, brown separatory funnel, combine all the extracts and use this extract as the test solution. Separately, weigh accurately about 10 mg of Methylergometrine Maleate RS, previously dried in a desiccator (silica gel) for 4 hours, dissolve in water and add water to make exactly 100 mL. Pipet 3.0 mL of this solution and transfer to a brown separator, proceed in the same manner as the preparation of the test solution and use this extract as the standard solution. To each total volume of the test solution and the standard solution, add 25.0 mL each of dilute 4-dimethylaminobenzaldehyde-iron (III) chloride TS, and after vigorous shaking for 5 minutes allow to stand for 30 minutes. Draw off the water layer, centrifuge and allow to stand for 1 hour. Perform the test with the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry, using dilute 4-dimethylaminobenzaldehyde-iron (III) chloride TS, as the blank. Determine the absorbances, A_T and A_S, of the subsequent solutions of the test solution and the standard solution at 545 nm, respectively.

Amount (mg) of methylergometrine maleate ($C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$) = Amount (mg) of Methylergometrine Maleate RS $\times \frac{A_T}{A_S} \times \frac{3}{100}$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Methylphenidate Hydrochloride



$C_{14}H_{19}NO_2 \cdot HCl$: 269.77

Methyl 2-phenyl-2-piperidin-2-ylacetate hydrochloride [*298-59-9*]

Methylphenidate Hydrochloride, when dried, contains not less than 98.0 % and not more than 100.5 % of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$).

Description Methylphenidate Hydrochloride appears as white fine crystals and is odorless.
Methylphenidate Hydrochloride is freely soluble in water or in methanol, soluble in ethanol (95) and practically insoluble in acetone or in chloroform.
A solution of Methylphenidate Hydrochloride changes the color of litmus paper to red.

Identification (1) Determine the infrared spectra of Methylphenidate Hydrochloride and Methylphenidate Hydrochloride RS, previously dried at 60 °C for 4 hours in vacuum as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) A solution of Methylphenidate Hydrochloride responds to the Qualitative Tests (2) for chloride.

Purity (1) ***Erythro[(R^*,S^*)] isomer***—Dissolve Methylphenidate Hydrochloride and Methylphenidate Hydrochloride erythro isomer RS in methanol to contain 50 mg per mL and 0.5 mg per mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 20 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28) (190 : 10 : 1) to a distance of about 15 cm and air-dry the plate. Spray evenly the plate with the detecting agent and then with 0.5 mol/L of sulfuric acid: the spot of the erythro isomer from the test solution is same in R_f value and is not larger and not more intense than that from the standard solution (not more than 1.0 %).

Detecting agent—Dissolve 0.7 g of bismuth subnitrate in 40 mL of a mixture of water and acetic acid (100) (4 : 1). Add 40 mL of potassium iodide solution (2 in 5) and then add 120 mL of acetic acid (100) and 250 mL of water.

(2) ***α-Phenyl-2-piperidineacetic acid hydrochloride***—Dissolve 0.4 g of Methylphenidate Hydrochloride, accurately weighed, in a solution of sodium hydroxide in methanol (1 in 2500) to make 10.0 mL and use this solution as the test solution (use immediately after preparation). Separately, dissolve a portion of α-phenyl-2-piperidineacetic acid hydrochloride RS in the solution of sodium hydroxide in methanol (1 in 2500) to obtain a solution having a known concentration of about 240 μg per mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and acetic acid (31) (65 : 25 : 5) to a distance of about 15 cm and air-dry the plate. Spray evenly the detecting agent and then hydrogen peroxide solution: any spot from the test solution having the same R_f value as the principal spot from the standard solution is not larger and is not more intense than that from the standard solution (not more than 0.6 %).

Detecting agent —Dissolve 0.85 g of bismuth subnitrate in 40 mL of a mixture of acetic acid (100) and water (1 : 4) (solution A). Dissolve 8.0 g of potassium iodide in 20 mL of water (solution B). Add 20 mL of acetic acid (100) to 10 mL of the mixture of solution A and B and water to make 100 mL.

(3) ***Heavy metals***—Proceed with 1.0 g of Methylphenidate Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 10 ppm).

Loss on Drying Not more than 0.5 % (1 g, in vacuum, 60 °C, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately 50 mg each of Methylphenidate Hydrochloride and Methylphenidate Hydrochloride RS, dissolve in the mobile phase to make exactly 100 mL and use these solutions as the test solution and the standard solution. Perform the test with 10 μL each

of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the peak areas, A_T and A_S, of methylphenidate in each solution.

$$\text{Amount (mg) of methylphenidate hydrochloride } (C_{14}H_{19}NO_2 \cdot HCl) = \text{Amount (mg) of Methylphenidate Hydrochloride RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 209 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Adjust the pH of a mixture of methanol and a 2.7 g/L solution of potassium dihydrogen phosphate-ammonium acetate (1 : 2) to 4.6 ± 0.1 with phosphoric acid.

Flow rate: 1.0 mL/minute

System suitability

System performance: Weigh accurately 5 mg of Erythro Isomer RS and 0.5 g of Methylphenidate Hydrochloride RS and dissolve in 1000 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peaks of the erythro isomer and methylphenidate is not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methylphenidate is not more than 2.0 %.

Time span of measurement: About twice as long as the retention time of methylphenidate.

Containers and Storage ***Containers***—Well-closed containers.

Methylphenidate Hydrochloride Tablets

Methylphenidate Hydrochloride Tablets contain not less than 93.0 % and not more than 107.0 % of the labeled amount of methylphenidate hydrochloride ($C_{14}H_{19}NO_2 \cdot HCl$: 269.77).

Method of Preparation Prepare as directed under Tablets, with Methylphenidate Hydrochloride.

Identification Place a portion of powdered Methylphenidate Hydrochloride Tablets, equivalent to about 50 mg of Methylphenidate Hydrochloride, in a 40-mL centrifuge tube, add 10 mL of chloroform, shake and centrifuge. Filter the clear supernatant liquid through a medium-sized sintered-glass funnel into a beaker and repeat the extraction with an additional 10 mL of chloroform. Evaporate the combined chloroform extracts on a water-bath to dryness. Agitate the dried residue with 2 mL of acetonitrile and filter the mixture through a small sintered-glass funnel. Wash the crystal with an additional 2 mL of acetonitrile and dry with the aid of suction. Determine the infrared spectra of the residue and Methylphenidate Hydrochloride RS as directed in the paste method under Infrared Spectrophometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Dissolution Test Perform the test with 1 tablet of Methylphenidate Hydrochloride Tablets at 100 resolutions per minute using 900 mL of water as the dissolution solution. Filter the dissolved solution after 45 minutes from the start of the test. Use the filtrate as the test solution and proceed as directed under the Assay. The dissolution rate of Methylphenidate Hydrochloride Tablets in 45 minutes is not less than 75 %.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Methylphenidate Hydrochloride Tablets. Weigh accurately a portion of the powder, equivalent to about 20 mg of Methylphenidate Hydrochloride, add about 70 mL of the mobile phase and shake by mechanical means for 15 minutes. Cool to room temperature and add the mobile phase to make exactly 100 mL. Filter this solution and discard the first 10 mL of the filtrate. Combine the subsequent 10.0 mL of the filtrate with 5.0 mL of the internal standard solution and use this solution as the test solution after shaking. Separately, weigh accurately about 20 mg of Methylphenidate Hydrochloride RS and dissolve in the mobile phase to make exactly 100 mL. To 5.0 mL of this solution, add 5.0 mL of the internal standard solution and use this solution as the standard solution. Perform the test with 50 μL each of the test solution and the standard solution as directed under Liquid Chromatography. Calculate the ratios, Q_T and Q_S, of the peak areas of Methylphenidate Hydrochloride to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of methylphenidate hydrochloride } (C_{14}H_{19}NO_2 \cdot HCl) = \text{Amount (mg) of Methylphenidate Hydrochloride RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—Weigh about 10 mg of Phenylephrine Hydrochloride and dissolve in mobile phase to make 25 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column, about 4.6 mm in

internal diameter and about 25 cm in length, packed with octadesylated silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Mobile phase: Dissolve 1.64 g of anhydrous sodium acetate in 900 mL of water, adjust the pH with acetic acid to 4.0 and add water to make 1000 mL. Add 300 mL of acetonitrile and 400 mL of methanol in 300 mL of this solution and mix.

Flow rate: 1.5 mL/minute.

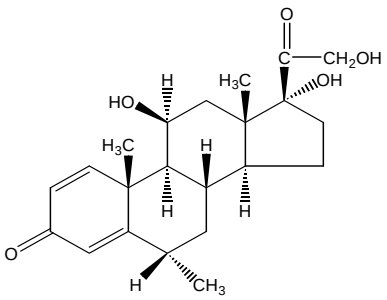
System suitability

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, phenylephrine and methylphenidate are eluted in this order with the resolution between their peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 50 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of methylphenidate is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Methylprednisolone



$C_{22}H_{30}O_5$: 374.47

(6*S*,8*S*,9*S*,10*R*,11*S*,13*S*,14*S*,17*R*)-11,17-Dihydroxy-17-(2-hydroxyacetyl)-6,10,13-trimethyl-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[a]phenanthren-3-one [*83-43-2*]

Methylprednisolone, when dried, contains not less than 96.0 % and not more than 104.0 % of methylprednisolone ($C_{22}H_{30}O_5$).

Description Methylprednisolone appears as white, crystalline powder and is odorless.

Methylprednisolone is sparingly soluble in methanol or in 1,4-dioxane, slightly soluble in ethanol (95) or in chloroform and practically insoluble in water or in ether.

Melting point—232 ~ 240 °C (with decomposition).

Identification (1) Add 2 mL of sulfuric acid to 2 mg of Methylprednisolone: a deep red color is observed with no fluorescence. Then add 10 mL of water to this solution: the color fades and a gray, flocculent precipitate is produced.

(2) Dissolve 10 mg of Methylprednisolone in 1 mL of methanol, add 1 mL of Fehling's TS and heat: a red precipitate is produced.

(3) Determine the absorption spectra of the solutions of Methylprednisolone and Methylprednisolone RS in methanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Specific Optical Rotation $[\alpha]_D^{20}$: +79 ~ +86° (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

Purity ***Related substances***—Dissolve 50 mg of Methylprednisolone in 5 mL of a mixture of chloroform and methanol (9 : 1) and use this solution as the test solution. Pipet 1.0 mL of this solution, add a mixture of chloroform and methanol (9 : 1) to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, ether, methanol and water (385 : 75 : 40 : 6) to a distance of about 12 cm and air-dry the plate. Then heat at 105 °C for 10 minutes, cool and spray evenly alkaline blue tetrazolium TS on the plate: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 1.0 % (0.5 g, 105 °C, 3 hours).

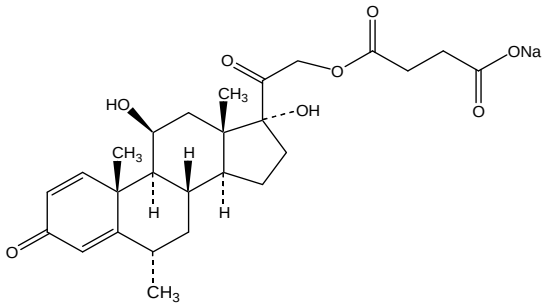
Residue on Ignition Not more than 0.2 % (0.2 g).

Assay Weigh accurately about 10 mg of Methylprednisolone, previously dried and dissolve in methanol to make exactly 100 mL. To 5.0 mL of this solution, add methanol to make exactly 50 mL and determine the absorbance, *A* at the wavelength of maximum absorption at about 243 nm, as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of methylprednisolone } (C_{22}H_{30}O_5) = \frac{A}{400} \times 10000$$

Containers and Storage ***Containers***—Tight containers.

Methylprednisolone Sodium Succinate



$C_{26}H_{33}NaO_8$: 496.53

Sodium 4-(2-((6*S*,8*S*,9*S*,10*R*,11*S*,13*S*,14*S*,17*R*)-11,17-dihydroxy-6,10,13-trimethyl-3-oxo-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[a]phenanthren-17-yl)-2-oxoethoxy)-4-oxobutanoate [*2375-03-3*]

Methylprednisolone Sodium Succinate contains not less than 97.0 % and not more than 103.0 % of methylprednisolone sodium succinate ($C_{26}H_{33}NaO_8$), calculated on the dried basis.

Description Methylprednisolone Sodium Succinate is a white, amorphous powder.
Methylprednisolone Sodium Succinate is very soluble in water or in ethanol (95), very slightly soluble in acetone, and practically insoluble in chloroform.
Methylprednisolone Sodium Succinate is hygroscopic.

Identification (1) Dissolve Methylprednisolone Sodium Succinate, previously dried, in methanol to make a concentration of 20 μg/mL. Perform the same procedure with Methylprednisolone Sodium Succinate RS. Determine the absorption spectra of both solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 0.1 g each of Methylprednisolone Sodium Succinate and Methylprednisolone Sodium Succinate RS in 10 mL of water. Add 1 mL of 3 mol/L hydrochloric acid, and extract immediately with 50 mL of chloroform. Filter the chloroform extract through cotton, evaporate on a steam bath to dryness, and dry in vacuum at 60 °C for 3 hours. Determine the infrared spectra of both residues so obtained as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Methylprednisolone Sodium Succinate responds to the Qualitative Tests (1) for sodium.

Specific Optical Rotation $[\alpha]_D^{20}$: +96 ~ +104° (0.1 g after drying, ethanol (95), 10 mL, 100 mm).

Purity ***Sodium***— Weigh accurately about 1.0 g of Methylprednisolone Sodium Succinate, previously dried, and dissolve in 75 mL of acetic acid (100) with gentle heating. Add 20 mL of 1,4-dioxane, and titrate with 0.1 N perchloric acid VS to a blue-green endpoint (indicator: 1 drop of methylrosaniline chloride TS). Perform a blank determination, and make any necessary correction (4.49 ~ 4.77 %).

Each mL of 0.1 mol/L perchloric acid VS
= 2.299 mg Na

Loss on Drying Not more than 3.0 % (1g, 105 °C, 3 hours)

Assay Weigh accurately about 0.1 g of Methylprednisolone Sodium Succinate, and dissolve it in ethanol (95) to make 200 mL. Pipet 5 mL of this solution, dilute with ethanol (95) to make 200 mL, and use this solution as the test solution. Separately, weigh accurately about 12.5 mg of Methylprednisolone Sodium Succinate RS, and dissolve in ethanol (95) to make exactly 100 mL. Pipet 5 mL of this solution, dilute with ethanol (95) to make 50 mL, and use this solution as the standard solution. Use ethanol (95) as the blank solution. To each of the glass-stoppered flasks containing 20 mL of the blank solution, the test solution, and the standard solution, add 2.0 mL of a solution prepared by dissolving 50 mg of blue tetrazolium in 10 mL of alcohol, and mix. Then, add to each flask 4.0 mL of a mixture of ethanol (95) and tetramethylammonium hydroxide TS (9:1). Mix, allow to stand in the dark for 90 minutes, and add 1.0 mL of acetic acid (100). Using the blank solution as control, determine the absorbances, A_T and A_S, at 525nm of the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry, and calculate the amount of Methylprednisolone Sodium Succinate.

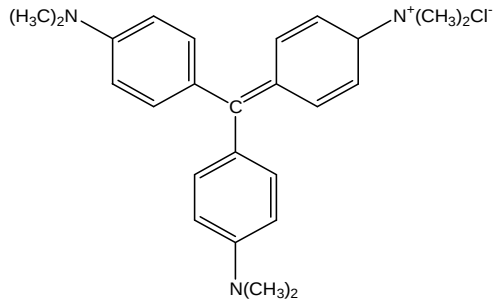
Amount (mg) of methylprednisolone sodium succinate ($C_{26}H_{33}NaO_8$) = $8.37 \times C \times \frac{A_T}{A_S}$

C: Concentration (μg/mL) of Methylprednisolone Sodium Succinate RS in the standard solution.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Methylrosanilinium Chloride



Crystal Violet $C_{25}H_{30}ClN_3$: 407.98

[4-[*bis*[4-(Dimethylamino)phenyl]methylidene] cyclohexa-2,5-dien-1-ylidene]-dimethylazanium chloride [*548-62-9*]

Methylrosanilinium Chloride is hexamethylpararosaniline chloride and is usually admixed with pentamethyl-pararosaniline chloride and tetramethylpararosaniline chloride.
Methylrosanilinium Chloride contains not less than 96.0 % and not more than 101.0 % of Methylrosanilinium Chloride [as hexamethylpararosaniline chloride ($C_{25}H_{30}ClN_3$)].

Description Methylrosanilinium Chloride is a green fragment having a metallic lust or a dark green powder, is odorless or has a slight ordor.
Methylrosanilinium Chloride is soluble in ethanol (95), sparingly soluble in water and practically insoluble in ether.

Identification (1) Take 1 mL of sulfuric acid and add 1 mg of Methylrosanilinium Chloride: Methylrosanilinium Chloride dissolves and shows an orange to red-brown color. To this solution, add water drop-wise: the color of the solution changes from brown through green to blue.

(2) Dissolve 20 mg of Methylrosanilinium Chloride in 10 mL of water, add 5 drops of hydrochloric acid and use this solution as the test solution. To 5 mL of the test solution, add tannic acid TS drop-wise: an intense blue precipitate is produced.

(3) Take 5 mL of the test solution obtained in (2), add 0.5 g of zinc powder and shake: the solution is decolorized. Place 1 drop of this solution on filter paper and apply 1 drop of ammonia TS adjacent to it: a blue color is observed at the zone of contact of both solutions.

Purity (1) ***Ethanol-insoluble substances***—Weigh accurately about 1 g of Methylrosanilinium Chloride, previously dried at 105 °C for 4 hours, heat with 50 mL of ethanol (95) under a reflux condenser for 15 minutes in a water-bath and filter the mixture through a tared glass filter (G4). Wash the residue on the filter with warm ethanol until the last washing does not show a purple color and dry at 105 °C for 2 hours: the weight of the residue is not more than 1.0 %.

(2) ***Heavy metals***—Proceed with 1.0 g of Methylrosanilinium Chloride according to Method 2 and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

(3) ***Zinc***—Take 0.10 g of Methylrosanilinium Chloride, add 0.1 mL of sulfuric acid and incinerate by ignition. After cooling, boil with 5 mL of dilute hydrochloric acid, 0.5 mL of dilute nitric acid and 4 mL of water, add 5 mL of ammonia TS, boil again and filter. To the filtrate, add 2 to 3 drops of sodium sulfide TS: no turbidity is produced.

(4) ***Arsenic***—Prepare the test solution with 0.40 g of Methylrosanilinium Chloride, according to Method 3 and perform the test (not more than 5 ppm).

Loss on Drying Not more than 7.5 % (1 g, 105 °C, 4 hours).

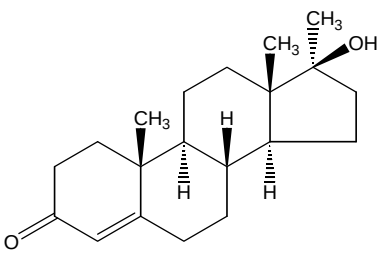
Residue on Ignition Not more than 1.5 % (0.5 g).

Assay Transfer about 0.4 g of Methylrosanilinium Chloride, accurately weighted, to a wide-mouthed Erlenmeyer flask, add 25 mL of water and 10 mL of hydrochloric acid, dissolve and add 50.0 mL of 0.1 mol/L titanium (III) chloride VS while passing a stream of carbon dioxide through the flask. Boil gently for 15 minutes, swirling the liquid frequently. Cool while passing a stream of carbon dioxide through the flask, titrate the excess titanium (III) chloride with 0.1 mol/L ferric ammonium sulfate VS until a faint, red color is observed (indicator: 5 mL of ammonium thiocyanate TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L titanium (III) chloride VS
= 20.399 mg of $C_{25}H_{30}ClN_3$

Containers and Storage ***Containers***—Tight containers.

Methyltestosterone



$C_{20}H_{30}O_2$: 302.45

(8*R*,9*S*,10*R*,13*S*,14*S*,17*S*)-17-Hydroxy-10,13,17-trimethyl-6,7,8,9,10,11,12,13,14,15,16,17-

dodecahydro-1*H*-cyclopenta[a]phenanthren-3(2*H*)-one [*58-18-4*]

Methyltestosterone, when dried, contains not less than 98.0 % and not more than 102.0 % of methyltestosterone ($C_{20}H_{30}O_2$).

Description Methyltestosterone appears as white to pale yellow crystals or crystalline powder and is odorless.
Methyltestosterone is freely soluble in methanol or in ethanol (95), soluble in acetone, sparingly soluble in ether and practically insoluble in water.

Identification (1) Determine the absorption spectra of the solutions of Methyltestosterone and Methyltestosterone RS in ethanol (95) (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Methyltestostrone and Methyltestosterone RS, previously dried, as directed n the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +79 ~ +85° (0.1 g, after drying, ethanol (95), 10 mL, 100 mm).

Melting Point 163 ~ 168 °C.

Purity ***Related substances***—Dissolve 40 mg of Methyltestosterone in 2 mL of ethanol and use this solution as the test solution. Pipet 1.0 mL of this solution, add ethanol (95) to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and diethylamine (19 : 1) to a distance of about 15 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 1.0 % (0.5 g, in vacuum, P_2O_5, 10 hours).

Residue on Ignition Not more than 0.1 % (0.5 g).

Assay Weigh accurately about 20 mg each of Methyltestosterone and Methyltestosterone RS, previously dried in a desiccator (in vacuum, phosphorous (V) oxide) for 10 hours, dissolve each in methanol to make exactly 200 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, add method to make 50 mL, and use these solutions as the test solution and the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine the ratios, Q_T and Q_S, of the peak area of methyltestosterone to that of the internal standard.

$$\text{Amount (mg)of Methyltestosterone } (C_{20}H_{30}O_2) = \text{Amount (mg) of Methyltestosterone RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of propyl parahydroxybenzoate in methanol (1 in 10000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 241 nm)

Column: A stainless steel column 6 mm in internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35 °C

Mobile phase: A mixture of acetonitrile and water (11:9)

Flow rate: Adjust the flow rate so that the retention time of methyltestosterone is about 10 minutes.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and methyltestosterone are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of methyltestosterone to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Methyltestosterone Tablets

Methyltestosterone Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of methyltestosterone ($C_{20}H_{30}O_2$: 302.45).

Method of Preparation Prepare as directed under Tablets, with Methyltestosterone.

Identification (1) Take a portion of powdered Methyltestosterone Tablets, equivalent to 10 mg of Methyltestosterone according to the labeled amount, add 50 mL of chloroform, shake for 30 minutes and

filter. Evaporate the filtrate to dryness, dissolve the residue in 10 mL of acetone and use this solution as the test solution. Separately, dissolve 10 mg of Methyltestosterone RS in 10 mL of acetone and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (9 : 1) to a distance of about 12 cm and air-dry the plate. Spray evenly dilute sulfuric acid on the plate and heat at 110 °C for 10 minutes: the spots from the test solution and the standard solution show the same R_f value.

Disintegration Test It meets the requirement.

Uniformity of Dosage Units It meets the requirement when the content uniformity test is performed according to the following method.

Take 1 tablet of Methyltestosterone Tablets, add 5 mL of water to disintegrate, add 50 mL of methanol and shake for 30 minutes. Add methanol to make exactly 100 mL and centrifuge. Measure exactly V mL of the clear supernatant liquid, add methanol to make exactly V' mL of a solution containing about 10 μg of methyltestosterone ($C_{20}H_{30}O_2$) per mL and use this solution as the test solution. Separately, weigh accurately about 10 mg of Methyltestosterone RS, previously dried in a desiccator (in vacuum, P_2O_5) for 10 hours, dissolve in 5 mL of water and 50 mL of methanol and then add methanol to make exactly 100 mL. Pipet 5.0 mL of this solution, add methanol to make exactly 50 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at the wavelength of a maximum absorption at about 241 nm as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of methyltestosterone } (C_{20}H_{30}O_2) = \text{Amount (mg) of Methyltestosterone RS} \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{10}$$

Assay Weigh accurately and powder not less than 20 Methyltestosterone Tablets. Weigh accurately a portion of the powder, equivalent to about 25 mg of methyltestosterone ($C_{20}H_{30}O_2$), add about 70 mL of methanol, mix by shaking for about 30 minutes, add methanol to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution and methanol to make exactly 50 mL, filter through a membrane filter (not exceeding 0.45 μm in a pore size), and use this solution as the test solution. Separately, weigh accurately about 20 mg of Methyltestosterone RS, previously dried in a desiccator (in vacuum, P_2O_5) for 10 hours and dissolve in methanol to make exactly 200 mL. Pipet 5 mL of this solution and 5 mL of the internal standard solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and calculate the ratios, Q_T and Q_S, of the peak area of methyltestosterone to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of methyltestosterone } (C_{20}H_{30}O_2) = \text{Amount (mg) of Methyltestosterone RS} \times \frac{Q_T}{Q_S} \times \frac{5}{4}$$

Internal standard solution—A solution of propyl paraoxybenzoiate in methanol (1 in 10000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 241 nm).

Column: A stainless steel column 6 mm in internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35 °C.

Mobile phase: A mixture of acetonitrile and water (11:9).

Flow rate: Adjust the flow rate so that the retention time of Methyltestosterone is about 10 minutes.

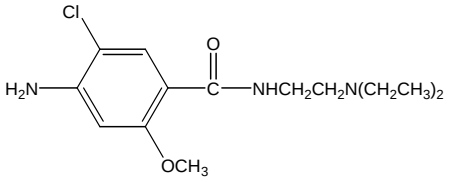
System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and Methyltestosterone are eluted in this order with the resolution between their peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of methyltestosterone to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Metoclopramide



$C_{14}H_{22}ClN_3O_2$: 299.80

4-Amino-5-chloro-*N*-[2-(diethylamino)ethyl]-2-methoxybenzamide [*364-62-5*]

Metoclopramide, when dried, contains not less than 99.0 % and not more than 101.0 % of metoclopramide ($C_{14}H_{22}ClN_3O_2$).

Description Metoclopramide appears as white crystals or a crystalline powder and is odorless.
Metoclopramide is freely soluble in acetic acid (100), soluble in methanol or in chloroform, sparingly soluble in ethanol (95), in acetic anhydride or in acetone, very slightly soluble in ether and practically insoluble in water.
Metoclopramide dissolves in dilute hydrochloric acid.

Identification (1) Dissolve 10 mg of Metoclopramide in 1 mL of dilute hydrochloric acid and 4 mL of water: the solution responds to the Qualitative Tests for primary aromatic amines.

(2) Dissolve 10 mg of Metoclopramide in 5 mL of dilute hydrochloric acid and 20 mL of water and to 5 mL of this solution, add 1 mL of Dragendorff's TS: an orange precipitate is produced.

(3) Dissolve 0.1 g each of Metoclopramide and Metoclopramide RS in 1 mL of 1 mol/L hydrochloric acid TS and dilute with water to make 100 mL. Determine the absorption spectra of the solutions, prepared by diluting 1 mL of both solutions with water to make 100 mL, as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting Point 146 ~ 149 °C.

Purity (1) ***Clarity and color of solution***— Dissolve 1.0 g of Metoclopramide in 10 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Metoclopramide as directed under Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Dissolve 1.0 g of Metoclopramide in 5 mL of 1 mol/L hydrochloric acid TS and use this solution as the test solution. Perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 0.10 g of Metoclopramide in 10 mL of methanol and use this solution as the test solution. Dilute 1 mL of this solution, exactly measured, with methanol to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol and ammonia solution (28) (19 : 1) to a distance of about 10 cm. Dry the plate, first in air and then at 80 °C for 30 minutes. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

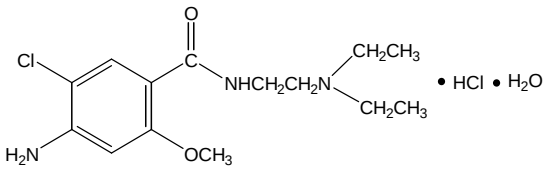
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.4 g of Metoclopramide, previously dried and dissolve in 50 mL of acetic acid (100), add 5 mL of acetic anhydride and warm for 5 minutes. After cool, titrate with 0.1 mol/L perchloric acid VS (indicator: 2 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.980 mg of $C_{14}H_{22}ClN_3O_2$

Containers and Storage ***Containers***—Well-closed containers.

Metoclopramide Hydrochloride Hydrate



Metoclopramide Hydrochloride
$C_{14}H_{22}ClN_3O_2 \cdot HCl \cdot H_2O$: 354.27

4-Amino-5-chloro-*N*-[2-(diethylamino)ethyl]-2-methoxybenzamide hydrate hydrochloride [*54143-57-6*]

Metoclopropamide Hydrochloride Hydrate contains not less than 98.0 % and not more than 101.0 % of metoclopropamide hydrochloride ($C_{14}H_{22}ClN_3O_2 \cdot HCl$: 336.26), calculated on the anhydrous basis.

Description Metoclopropamide Hydrochloride Hydrate appears as white crystalline powder and is orderless or has a faint odor.
Metoclopropamide Hydrochloride Hydrate is very soluble in water, freely soluble in ethanol (95), sparingly soluble in chloroform and practically insoluble in ether.

Identification (1) Dissolve 50 mg of Metoclopropamide Hydrochloride Hydrate in 5 mL of water and add 5 mL of 4-dimethylaminobenzaldehyde in 1 mol/L hydrochloric acid (1 in 100): an orange color is observed.

(2) The R_f value of the principal spot in the chromatogram of the identification solution corresponds to that of the standard solution A, as directed under the Purity test.

(3) Determine the infrared spectrum of Metoclopropamide Hydrochloride Hydrate and Metoclopropamide Hydrochloride Hydrate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity (1) ***Heavy metals***—Dissolve 2.5 g of Metoclopramide Hydrochloride Hydrate in 25 mL of freshly boiled and cooled water, and use 12 mL of this solution as the test solution. Separately, prepare 2 mL of standard lead solution in the same manner as the test solution. To 10 mL of this solution, add 2 mL of the test solution and use this solution as the control solution. Separately, add 2 mL of the test solution to 10 mL of water and use this solution as the blank solution. To 12 mL each of the test solution, the control solution and the blank solution, add 2 mL of pH 3.5 acetate buffer, mix, add 1.2 mL of thioacetamide TS and mix immediately. Allow to stand for 2 minutes: the test solution has no more color than the control solution (not more than 20 ppm).

System suitability: The control solution shows a faint brown color compared to the blank solution.

(2) ***Related substances***—Dissolve an accurately weighed quantity of Metoclopropamide Hydrochloride Hydrate in methanol to obtain a solution containing 50 mg per mL and use this solution as the test solution. Dilute a volume of the test solution quantitatively with methanol to obtain a solution containing 0.5 mg per mL and use this solution as the identification solution. Dilute a volume of the test solution quantitatively with methanol to obtain three standard solutions, designated below by letters, having the following concentrations:

Standard solutions	Dilution	Concentration (μg/mL)	Ratio (%) of the sample
A	(1 in 4)	250	0.5
B	(3 in 20)	150	0.3
C	(1 in 20)	50	0.1

Perform the test with the test solution, the identification solution and the standard solutions, A, B, and C as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution, the identification solution and the standard solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, toluene and strong ammonia water (140 : 60 : 20 : 1) to a distance of about 15 cm and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot from the test solution is not larger and is not more intense than the principal spot from the standard solution A (0.5 %) and the sum of the intensities all secondary spots from the test solution is not more than 1.0 %.

Water 4.5 ~ 6.0 % (0.5 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

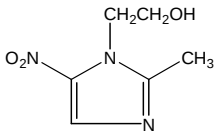
Assay Weigh accurately about 0.3 g of Metoclopropamide Hydrochloride Hydrate, add 10 mL of mercuric acetate and 2 mL of acetic anhydride and allow to stand for 3 hours. Add 80 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 33.626 mg of $C_{14}H_{22}ClN_3O_2 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Metronidazole



$C_6H_9N_3O_3$: 171.15

2-(2-Methyl-5-nitroimidazol-1-yl)ethanol [*443-48-1*]

Metronidazole, when dried, contains not less than 99.0 % and not more than 101.0 % of metronidazole ($C_6H_9N_3O_3$).

Description Metronidazole appears as white to pale yellow crystals or crystalline powder, is odorless and has a slightly bitter taste.
Metronidazole is freely soluble in acetic acid (100), sparingly soluble in methanol, in ethanol (95) or in acetone, slightly soluble in water and very slightly soluble in ether.
Metronidazole dissolves in dilute hydrochloric acid.
Metronidazole is affected by light.

Identification (1) Determine the absorption spectra of the solutions of Metronidazole and Metronidazole RS in 0.1 mol/L hydrochloric acid TS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Metronidazole and Metronidazole RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 159 ~ 163 °C.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Metronidazole according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***2-Methyl-5-nitroimidazole***—Dissolve 0.10 g of Metronidazole in acetate to make exactly 10 mL and use this solution as the test solution. Separately, dissolve 20 mg of 2-methyl-5-nitroimidazole RS in acetone to make exactly 20 mL, then pipet 5.0 mL of this solution, add acetone to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 20 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Immediately develop the plate with a mixture of acetone, water and ethyl acetate (8 : 1 : 1) to a distance of about 15 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot form the test solution corresponding to the spot from the standard solution is not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, silica gel, 24 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.2 g of Metronidazole, previously dried and dissolve in 30 mL of acetic acid (100). Titrate with 0.1 mol/L perchloric acid VS (indicator: 0.5 mL of 1-naphtholbenzeine TS) until the color of the solution changes from orange to green. Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 17.115 mg of $C_6H_9N_3O_3$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Metronidazole Tablets

Metronidazole Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of metronidazole ($C_6H_9N_3O_3$: 171.15).

Method of Preparation Prepare as directed under Tablets with Metronidazole.

Identification (1) Weigh accurately an amount of powdered Metronidazole Tablets, equivalent to about 0.1 g of metronidazole according to the labeled amount, and add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Allow to stand for 30 minutes with occasional vigorous shaking then centrifuge. To 1 mL of the clear supernatant liquid, add 0.1 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 275 nm and 279 nm.

(2) Powder Metronidazole Tablets, weigh a quantity equivalent to 0.20 g of Metronidazole according to the labeled amount, shake vigorously with 20 mL of acetone for 10 minutes, centrifuge, and use the clear supernatant liquid as the test solution. Separately, dissolve 0.10 g of Metronidazole RS in 10 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL of each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate immediately with a mixture of acetone, water and ethyl acetate (8 : 1 : 1) to a distance of 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the R_f value of the principal spots obtained from the test solution and the standard solution is the same.

Dissolution Test Perform the test with 1 tablet of Metronidazole Tablets at 50 revolutions per minute according to Method 2 under Dissolution Test, using 900 mL of water as the dissolution solution. Take not less than 20 mL of the dissolved solution after 90 minutes from the start of the test and filter through a membrane filter with pore size of not more than 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make V' mL so that each mL contains about 11 μg of metronidazole according to the labeled amount and use this solution as the test solution. Separately, weigh accurately about 22 mg of Metronidazole RS, previously dried in vacuum with silica gel for 24 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 320 nm of the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry. The dissolution rate of Metronidazole Tablets in 90 minutes is not less than 70 %.

Dissolution rate (%) with respect to the labeled amount of metronidazole ($C_6H_9N_3O_3$)

$$= W_S \times \frac{V'}{V} \times \frac{A_T}{A_S} \times \frac{1}{C} \times 45$$

W_S: Amount (mg) of Metronidazole RS

C: Labeled amount (mg) of metronidazole in 1 tablet

Uniformity of Dosage Units It meets the requirement when the content uniformity test is performed according to the following method.

To 1 tablet of Metronidazole Tablets, add 25 mL of a

mixture of wate and methanol (1 : 1), shake vigorously for 25 minutes, and add the mixture of water and methanol (1 : 1) to make exactly 50 mL. Pipet 5 mL of this solution, and add a mixture of water and methanol (1 : 1) to make exactly 100 mL. Filter the solution through a membrane filter with a pore size of 0.45 μm, discard the first 3 mL of the filtrate, and use the subsequent filtrate as the test solution. Hereinafter, proceed as directed under the Assay.

$$\text{Amount (mg) of metronidazole } (C_6H_9N_3O_3) = \text{Amount (mg) of Metronidazole RS} \times \frac{A_T}{A_S} \times 10$$

Assay Weigh accurately and powder not less than 20 Metronidazole Tablets. Weigh accurately a portion of the powder, equivalent to about 0.25 g of metronidazole ($C_6H_9N_3O_3$), add 25 mL of a mixture of water and methanol (1 : 1), shake vigorously for 10 minutes and add a mixture of water and methanol (1 : 1) to make exactly 50 mL. Pipet 5 mL of this solution and add a mixture of water and methanol (4 : 1) to make exactly 100 mL. Filter through a membrane filter with a pore size of not more than 0.45 μm, discard the first 3 mL of the filtrate and use the subsequent filtrate as the test solution. Separately, weigh accurately about 25 mg of Metronidazole RS, previously dried in vacuum with silica gel for 24 hours, dissolve in a mixture of water and methanol (4 : 1) to make exactly 100 mL and use this solution as the standard solution. Perform the test with exactly 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the peak areas, A_T and A_S, of metronidazole in each solution.

$$\text{Amount (mg) of metronidazole } (C_6H_9N_3O_3) = \text{Amount (mg) of Metronidazole RS} \times \frac{A_T}{A_S} \times 10$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 320 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: A mixture of water and methanol (80 : 20).

Flow rate: Adjust the flow rate so that the retention time of metronidazole is about 5 minutes..

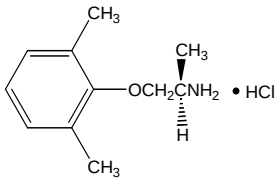
System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of metronidazole is not less than 3000 with the symmetry factor being not more than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of Metronidazole is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Mexiletine Hydrochloride



and enantiomer

$C_{11}H_{17}NO \cdot HCl$: 215.72

(*RS*)-1-(2,6-Dimethylphenoxy)propan-2-amine hydrochloride [*5370-01-4*]

Mexiletine Hydrochloride, when dried, contains not less than 98.0 % and not more than 102.0 % of mexiletine hydrochloride ($C_{11}H_{17}NO \cdot HCl$).

Description Mexiletine Hydrochloride appears as white powder.

Mexiletine Hydrochloride is freely soluble in water or in ethanol (95), slightly soluble in acetonitrile, and practically insoluble in ether.

A solution of Mexiletine Hydrochloride (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectra of the solutions of Mexiletine Hydrochloride and Mexiletine Hydrochloride RS in 0.01 mol/L hydrochloric acid TS (1 in 2000) as directed under the the Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Mexiletine Hydrochloride and Mexiletine Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears between the spectra, recrystallize the sample from ethanol (95), filter, dry the crystals, and repeat the test with the crystals.

(3) A solution of Mexiletine Hydrochloride (1 in 100) responds to the Qualitative Tests (2) for chloride.

Melting Point 200 ~ 204 °C.

pH Dissolve 1.0 g of Mexiletine Hydrochloride in 10 mL of water. The pH of this solution is between 3.8 and 5.8.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Mexiletine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) ***Heavy Metals***—Proceed with 2.0 g of Mexiletine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Dissolve 20 mg of Mexiletine Hydrochloride in 20 mL of the mobile phase, and use this solution as the test solution. Pipet 1.0 mL of the test solution, add the mobile phase to make exactly 250 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine each peak area of the test solution and the standard solution by automatic integration method: each peak area of the peaks other than the principal peak from the test solution is not larger than the principal peak area from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, flow rate, and selection of column: Proceed as directed in the operating conditions in the Assay under Mexiletine Hydrochloride.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of mexiletine obtained from 20 μL of the standard solution is between 5 mm and 10 mm.

Time span of measurement: About 3 times as long as the retention time of mexiletine after peaks of the solvent.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 20 mg each of Mexiletine Hydrochloride and Mexiletine Hydrochloride RS, previously dried, and dissolve each in the mobile phase to make exactly 20 mL. Pipet 5.0 mL each of these solutions, add 5.0 mL of the internal standard solution, then add the mobile phase to make exactly 100 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine the ratios, Q_T and Q_S, of the peak area of mexiletine to that of the internal standard, for the test solution and the standard solution, repectively.

Amount (mg) of mexiletine hydrochloride ($C_{11}H_{17}NO \cdot HCl$) = Amount (mg) of Mexiletine Hydrochloride RS $\times \dfrac{Q_T}{Q_S}$

Internal standard solution—A solution of phenetylamine hydrochloride in the mobile phase (3 in 5000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column, about 4 mm in internal diameter and 15 cm to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (about 7 μm in particle diameter).

Column temperature: A constant temperature of about 30 °C.

Mobile phase: Dissolve 2.5 g of sodium lauryl sulfate and 3 g of monobasic sodium phosphate in 600 mL of water, and add 420 mL of acetonitrile.

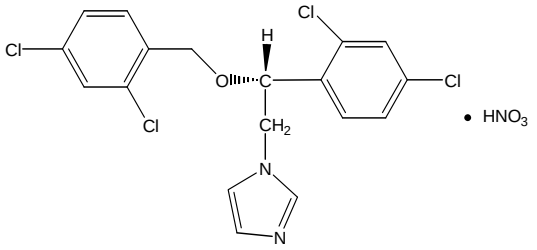
Flow rate: Adjust the flow rate so that the retention time of mexiletine is about 6 minutes.

Selection of column: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard and mexiletine are eluted in this order with the resolution between their peaks being not less than 9.0.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Miconazole Nitrate



and enantiomer

$C_{18}H_{14}Cl_4N_2O \cdot HNO_3$: 479.14

(*RS*)-1-[2-(2,4-Dichlorophenyl)-2-[(2,4-dichlorophenyl)methoxy]ethyl]imidazole; nitric acid [*22832-87-7*]

Miconazole Nitrate, when dried, contains not less than 98.5 % and not more than 101.0 % of miconazole nitrate ($C_{18}H_{14}Cl_4N_2O \cdot HNO_3$).

Description Miconazole Nitrate is a white crystalline powder.

Miconazole Nitrate is freely soluble in *N,N*-dimethylformamide, sparingly soluble in methanol, slightly soluble in ethanol (95), in acetone or in acetic acid (100)

and very slightly soluble in water or in ether.

Melting point—About 180 °C (with decomposition).

Identification (1) Take 2 mL of a solution of Miconazole Nitrate in methanol (1 in 100) and add 2 mL of Reinecke salt TS: a pale red precipitate is formed.

(2) Determine the absorption spectra of the solutions of Miconazole Nitrate and Miconazole Nitrate RS in methanol (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Perform the test with a solution of Miconazole Nitrate in methanol (1 in 100) as directed under the Flame Coloration Test (2): a green color appears.

(4) A solution of Miconazole Nitrate in methanol (1 in 100) responds to the Qualitative Tests for nitrate.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Miconazole Nitrate in 100 mL of methanol: the solution is clear and colorless.

(2) ***Chloride***—Dissolve 0.10 g of Miconazole Nitrate in 6 mL of dilute nitric acid and *N,N*-dimethylformamide to make 50 mL. Perform the test. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS, add 6 mL of dilute nitric acid and *N,N*-dimethyl-formamide to make 50 mL (not more than 0.09 %).

(3) ***Heavy metals***—Proceed with 1.0 g of Miconazole Nitrate according to Method 2 and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(4) ***Arsenic***—Prepare the test solution with 1.0 g of Miconazole Nitrate according to Method 3 and perform the test (not more than 2 ppm).

(5) ***Related substances***—Dissolve 0.10 g of Miconazole Nitrate in 10 mL of methanol and use this solution as the test solution. Pipet 1.0 mL of the test solution, add methanol to make exactly 20 mL, pipet 1.0 mL of this solution, add methanol to make exactly 20 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 50 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of n-hexane, chloroform, methanol and ammonia solution (28) (60 : 30 : 10 : 1) to a distance of about 12 cm and air-dry the plate. Expose the plate in iodine vapor for 20 minutes: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, silica gel, 60 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

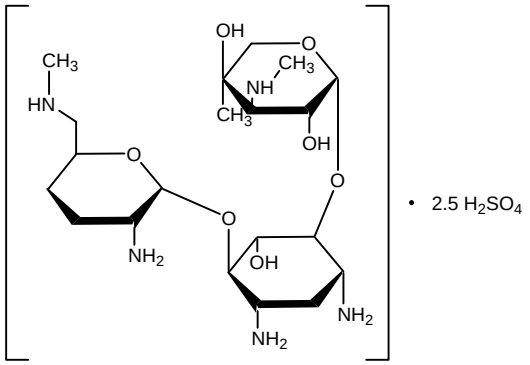
Assay Weigh accurately about 0.35 g of Miconazole Nitrate, previously dried, dissolve in 50 mL of acetic acid (100) by warming, cool and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 47.91 mg of $C_{18}H_{14}Cl_4N_2O \cdot HNO_3$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Micronomicin Sulfate



$(C_{20}H_{41}N_5O_7)_2 \cdot 2.5H_2SO_4$: 708.77

(2*R*,3*R*,4*R*,5*R*)-2-[(1*S*,2*S*,3*R*,4*S*,6*R*)-4,6-Diamino-3-[(2*R*,3*R*,6*S*)-3-amino-6-(methylaminomethyl)oxan-2-yl]oxy-2-hydroxycyclohexyl]oxy-5-methyl-4-(methylamino)oxane-3,5-diol; sulfuric acid [*52093-21-7*, Micronomicin]

Micronomicin Sulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of *Micromonospora sagamiensis*.
Micronomicin Sulfate contains not less than 590 μg (potency) and not more than 660 μg (potency) per mg of micronomicin ($C_{20}H_{41}N_5O_7$: 463.57), calculated on the anhydrous basis.

Description Micronomicin Sulfate appears as white to pale yellowish white crystal powder.
Micronomicin Sulfate is very soluble in water, sparingly soluble in ethylene glycol and practically insoluble in methanol or in ethanol (99.5).
Micronomicin Sulfate is hygroscopic.

Identification (1) Dissolve 50 mg (potency) each of Micronomicin Sulfate and Micronomicin Sulfate RS in 10 mL of water and use these solutions as the test solution and the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the

standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), 1-butanol and ammonia solution (28) (10 : 8 : 7) to a distance of about 10 cm and air-dry the plate. Spray evenly a solution of ninhydrin in a mixture of acetone and pyridine (25 : 1) (1 in 500) and heat at 100 °C for 10 minutes: the spots obtained from the sample solution and the standard solution are red-purple to red-brown and their R_f values are the same.

(2) To 5 mL of a solution of Micronomicin Sulfate (1 in 500), add 1 mL of barium chloride TS: a white precipitate is formed and it does not dissolve by addition of dilute nitric acid.

Specific Optical Rotation $[\alpha]_D^{20}$: +110 ~ +130° (0.25 g calculated on the anhydrous basis, water, 25 mL, 100 mm)

pH The pH of a solution obtained by dissolving 1.0 g of Micronomicin Sulfate in 10 mL of water is between 3.5 and 5.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.5 g of Micronomicin Sulfate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) ***Heavy metals***—Proceed with 1.0 g of Micronomicin Sulfate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution. (not more than 20 ppm).

(3) ***Related substances***—Dissolve 0.40 g of Micronomicin Sulfate in 10 mL of water and use this solution as the test solution. Pipet 1 mL of this solution, add water to make exactly 200 mL and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), 1-butanol and ammonia solution (28) (10 : 8 : 7) to a distance of about 10 cm and air-dry the plate. Spray evenly a solution of ninhydrin in a mixture of acetone and pyridine (25 : 1) (1 in 500) and heat at 100 °C for 10 minutes: the spot other than the principal spot obtained from the test solution is not more intense than the spot from the standard solution.

Water Not more than 10.0 % (0.2 g, volumetric titration, back titration, use a mixture of methanol for water determination and ethylene glycol for water determination (1:1) instead of methanol for water determination.)

Sterility Test It meets the requirement, when Micronomicin Sulfate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.50 EU/mg (potency) of micronomicin, when Micronomicin Sulfate is used in a sterile preparation.

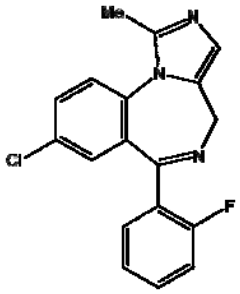
Assay ***The Cylinder-plate method*** (1) Agar media for seed and base layer- Use the culture medium in I 2 1) (1) ① under Microbial Assay for Antibiotics.

(2) Test organism- *Bacillus subtilis* ATCC 6633

(3) Weigh accurately an amount of Micronomicin Suflate, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer for antibiotics (pH 8.0) to make exactly 20 mL. Pipet a suitable amount of this solution, dilute with 0.1 mol/L phosphate buffer for antibiotics (pH 8.0) so that each mL contains 2 μg (potency) and 0.5 μg (potency) and use these solutions as the high concentration test solution and the low concentration test solution, respectively. Separately, weigh accurately an amount of Micronomicin Sulfate RS, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer for antibiotics (pH 8.0) to make exactly 20 mL and use this solution as the standard stock solution. Keep the standard stock solution at a temperature between 5 °C and 15 °C and use within 30 days. Pipet a suitable amount of the standard stock solution before use, dilute with 0.1 mol/L phosphate buffer for antibiotics (pH 8.0) so that each mL contains 2 μg (potency) and 0.5 μg (potency) and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively. Perform the test with these solutions as directed in I 8 under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Midazolam



$C_{18}H_{13}ClFN_3$: 325.77

8-Chloro-6-(2-fluorophenyl)-1-methyl-4*H*-imidazo [1,5-a][1,4]benzodiazepine [*59467-70-8*]

Midazolam contains not less than 98.5 % and not more than 101.5 % of midazolam ($C_{18}H_{13}ClFN_3$), calculated on a dried basis.

Description Midazolam is a whilte or yellow, crystalline powder.
Midazolam is freely soluble in acetone or in ethanol (95), and soluble in methanol, and practically insoluble in water.

Identification (1) Determine the infrared spectra of Midazolam and Midazolam RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2)The principal spot from the test solution (2) and the principal spot from the standard solution (2), as obtained in the test of the related substances in the Purity show same R_f value.

(3) Mix 90 mg of Midazolam with 0.30 g of anhydrous sodium carbonate and ignite in a crucible until an almost white residue is obtained (normally in less than 5 minutes). Allow to cool, dissolve the residue in 5 mL of dilute nitric acid, and filter. Add 1.0 mL of the filtrate to a freshly prepared mixture of 0.1 mL of alizarin S TS and 0.1 mL of zirconyl nitrate TS. Mix, and allow to stand for 5 minutes. This solution is yellow and the blank solution prepared in the same manner is red.

(4) To 1 mL of the filtrate obtained in the Indentification test (2), add 1 mL of water. The solution responds to the Qualitative Tests (1) for chloride.

Melting Point 161 ~ 164 °C.

Purity (1) ***Clarity of solution***—The solution obtained by dissolving 0.1 g of Midazolam in 0.1 mol/L hydrochloric acid TS is clear.

(2) ***Related substances***—(i) Dissolve 0.2 g of Midazolam in ethanol (95) to make 5 mL, and use this solution as the test solution (1). Dilute 1.0 mL of this solution with ethanol (95) to 50 mL, and use this solution as the test solution (2). To 1.0 mL of the test solution (1), add ethanol (95) to make 10 mL. Pipet 1.0 mL of this solution, dilute with ethanol (95) to 100 mL, and use this solution as the standard solution (1). Dissolve 8 mg of Midazolam RS in ethanol (95) to make 10 mL, and use this solution as the standard solution (2). Separately, dissolve 8 mg each of Midazolam RS and chlorodiazepoxide in ethanol (95) to make 10 mL, and use this solution as the standard solution (3). Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solutions on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop with a mixture of ethyl acetate, methanol, water, and acetic acid (100) (80:20:15:2) to a distance of about 12 cm, and dry the plate in air. Examine in ultraviolet light at 254 nm. Any spot due to related substances from the test solution (1) is not more intense than the spot from the standard solution (1) (not more than 0.1 %). This test passes system suitability when the chromatogram obtained with the standard solution (3) shows 2 clearly separated spots.

(ii) Dissolve about 50 mg of Midazolam in methanol to make 50 mL and use this solution as the test solution. To 1 mL of this solution, add methanol to make 100 mL. To 1 mL of this solution, add methanol to make 10 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine each peak area of each solution by the automatic integration method: the peak areas of related substance I {(6*RS*)-8-chloro-6-(2-fluorophenyl)-1-methyl-5,6-dihydro-4*H*-imidazo[1.5-α][1,4]benzodiazepine} and related substance II {8-chloro-1-methyl-6-phenyl-4*H*-imidazo[1.5-α][1,4]benzodiazepine} obtained from the test solution are not larger than the area of the principal peak from the standard solution (0.1 %), and the peak area of related substance III {(6RS)-8-chloro-6-(2-fluorophenyl)-1-methyl-6*H*-imidazo[1.5-α][1,4]benzodiazepine} is not larger than 2 times the area of the principal peak from the standard solution (0.2 %). The peak area of related substances other than related substances I, II and III obtained from the test solution is not larger than the area of the principal peak from the standard solution (0.1 %). The total area of related substances is not larger than 3 times the area of the principal peak obtained from the standard solution (0.3 %). Disregard any peaks with an area not larger than 0.5 times the area of the principal peak from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.0 mm in internal diameter and about 25 cm in length, packed with octylsilyl silica gel for liquid chromatography (3 μm in particle diameter).

Mobile phase: Dissolve 7.7 g of ammonium acetate in 1000 mL of water, dissolve 4 g of tetrabutylammonium hydroxide in 1000 mL of water, mix these solutions and adjust the pH to 5.3 with acetic acid. To 440 mL of this solution, add 560 mL of methanol.

Flow rate: 1.0 mL/minute

Time span of measurement: About 2.5 times as long as the retention time of midazolam.

Relative retention time: When the procedure is run with 5 μL each of the test solution and the standard solution, the relative retention times of related substances I, II and III with respect to the peak of midazolam are about 0.9, about 1.2 and about 2.2, respectively.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

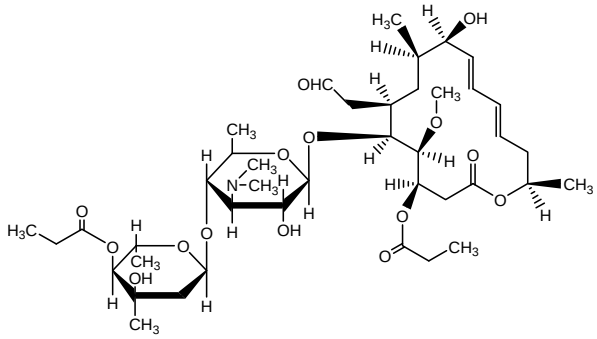
Assay Dissolve 0.120 g of Midazolam in 30 mL of acetic acid (100), add 20 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry).

Each mL of 0.1 mol/L perchloric acid VS
= 16.29 mg of $C_{18}H_{13}ClFN_3$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Midecamycin



$C_{41}H_{67}NO_{15}$: 813.97

[(4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-6-[(2*S*,-3*R*,4*R*,5*S*,6*R*)-4-(Dimethylamino)-3-hydroxy-5-[(2S,4R,5S,6S)-4-hydroxy-4,6-dimethyl-5-propanoyloxyoxan-2-yl]oxy-6-methyloxan-2-yl]oxy-10-hydroxy-5-methoxy-9,16-dimethyl-2-oxo-7-(2-oxoethyl)-1-oxacyclohexadeca-11,13-dien-4-yl] propanoate [*35457-80-8*]

Midecamycin contains not less than 950 μg (potency) and not more than 1020 μg (potency) per mg of midecamycin ($C_{41}H_{67}NO_{15}$: 813.97), calculated on the dried basis.

Description Midecamycin appears as white crystalline powder, is odorless and has a bitter taste.
Midecamycin is very soluble in methanol, freely soluble in ethanol (95) or in ethyl acetate, sparingly soluble in ether and very slightly soluble in water.

Identification (1) Dissolve 2 mg (potency) of Midecamycin in 5 mL of sulfuric acid: a red-brown color develops.

(2) Dissolve 1 mg (potency) of Midecamycin in 50 mL of methanol and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 230 nm and 233 nm.

Melting Point 153 ~ 158 °C

Purity ***Heavy metals***—Proceed with 1.0 g of Midecamycin according to Method 2 and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

Loss on Drying Not more than 2.0 % (1.0 g, 0.7 kPa, 60 °C, 3 hours)

Residue on Ignition Not more than 0.2 % (1 g)

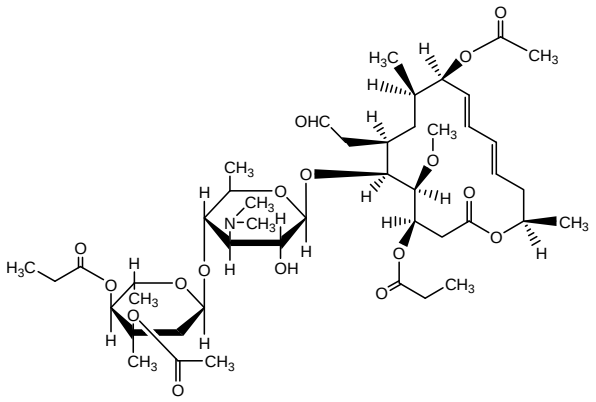
Assay ***The Cylinder-plate method*** (1) Agar media for seed and base layer- Use the culture medium in I 2 1) (1) ① under Microbial Assay for Antibiotics.

(2) Test organism- *Bacillus subtilis* ATCC 6633

(3) Weigh accurately about 0.2 g (potency) of Midecamycin, dissolve in 10 mL of methanol and add sterile purified water to make a solution containing 400 μg (potency) per mL. Pipet a suitable amount of this solution, dilute with 0.1 mol/L phosphate buffer (pH 8.0) so that each mL contains 20.0 and 5.0 μg (potency) and use these solutions as the high concentration test solution and the low concentration test solution, respectively. Separately, weigh accurately about 20 mg (potency) of Midecamycin RS, dissolve in 10 mL of methanol and add sterile purified water to make a standard stock solution containing 400 μg (potency) per mL. Keep this standard stock solution at not exceeting 5 °C and use within 7 days. Pipet a suitable amount of the standard stock solution, dilute with 0.1 mol/L phosphate buffer (pH 8.0) so that each mL contains 20.0 and 5.0 μg and (potency) and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively. Perform the test with these solutions as directed in I 8 under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Midecamycin Acetate



$C_{45}H_{71}NO_{17}$: 898.04

[(4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-10-Acetyloxy-6-[(2*S*,3*R*,4*R*,5*S*,6*R*)-5-[(2*S*,4*R*,5*S*,6*S*)-4-acetyloxy-4,6-dimethyl-5-propanoyloxyoxan-2-yl]oxy-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-5-methoxy-9,16-dimethyl-2-oxo-7-(2-oxoethyl)-1-oxacyclohexadeca-11,13-dien-4-yl] propanoate [*55881-07-7*]

Midecamycin Acetate is a derivative of midecamycin. Midecamycin Acetate contains not less than 950 μg (potency) and not more than 1010 μg (potency) per mg

of midecamycin acetate ($C_{45}H_{71}NO_{17}$: 898.04), calculated on the dried basis.

Description Midecamycin Acetate appears as white crystals or crystalline powder.
Midecamycin Acetate is sparingly soluble in methanol, slightly soluble in ethanol (95) and practically insoluble in water.

Identification (1) Determine the absorption spectra of the solutions of Midecamycin Acetate and Midecamycin Acetate RS (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Midecamycin Acetate and Midecamycin Acetate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity ***Heavy metals***—Proceed with 1.0 g of Midecamycin Acetate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

Loss on Drying Not more than 2.0 % (1.0 g, in vacuum, not exceeding 0.67 kPa, 60 °C, 3 hours).

Residue on Ignition Not more than 0.2 % (1 g)

Assay Weigh accurately about 50 mg (potency) each of Midecamycin Acetate and Midecamycin Acetate RS, dissolve each in the mobile phase to make exactly 50 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following operating conditions and determine the peak areas, A_T and A_S, of midecamycin acetate in the test solution and the standard solution.

$$\text{Amount [μg (potency)] of midecamycin acetate per mg of Midecamycin Acetate} = \frac{A_T}{A_S} \times \frac{\text{Amount [}\mu\text{g/mg (potency)] in the amount of Midecamycin Acetate RS taken}}{\text{Amount (mg) of Midecamycin Acetate taken}}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm)

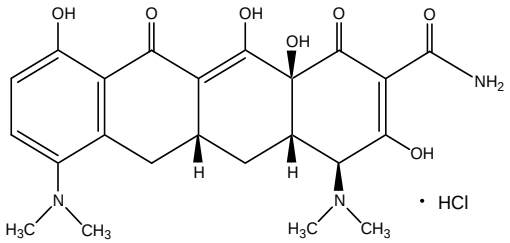
Column: A stainless steel column about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Adjust the pH of a mixture of 0.01 nol/L potassium dihydrogen phosphate TS, 0.01 mol/L dipotassium hydrogen phosphate TS and acetonitrile (250:100:400) to 3.0 with phosphoric acid.

Flow rate: 1.0 mL/minute

Containers and Storage ***Containers***—Tight containers.

Minocycline Hydrochloride



$C_{23}H_{27}N_3O_7 \cdot HCl$: 493.94

(4*S*,4*aS*,5*aR*,12*aS*)-4,7-Bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide hydrochloride [*13614-98-7*]

Minocycline Hydrochloride is the hydrochloride of a derivative of tetracycline.
Minocycline Hydrochloride contains not less than 890 μg (potency) and not more than 950 μg (potency) per mg of minocycline ($C_{23}H_{27}N_3O_7$: 457.48), calculated on the anhydrous basis.

Description Minocycline Hydrochloride appears as yellow crystalline powder.
Minocycline Hydrochloride is freely soluble in *N,N*-dimethylformamide, soluble in methanol, sparingly soluble in water and slightly soluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Minocycline Hydrochloride in a solution of hydrochloric acid in methanol (19 in 20000) (1 in 62500) as directed under Ultraviolet-visible Spectrophotometry and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Minocycline Hydrochloride and Minocycline Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Minocycline Hydrochloride (1 in 100) responds to the Qualitative Tests (2) for chloride.

Crystallinity Test It meets the requirement.

pH The pH of a solution obtained by dissolving 1 g of Minocycline Hydrochloride in 100 mL of water is between 3.5 and 4.5.

Absorbance $E^{1\%}_{1\,cm}$ (358 nm): 296 ~ 328 (8 mg, 0.01 mol/L hydrochloric acid-methanol TS, 500 mL)

Purity (1) ***Clarity of solution***—Dissolve 1.0 g of Minocycline Hydrochloride in 100 mL of water: the solution is clear. When the test is performed as directed under Ultraviolet-visible Spectrophotometry within 1 hour after preparation of this solution, the absorbance of the solution at 560 nm is not more than 0.06.

(2) ***Heavy metals***—Proceed with 0.5 g of Minocycline Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 2.5 mL of standard lead solution (not more than 50 ppm).

(3) ***Related substances***—Dissolve 50 mg of Minocycline Hydrochloride in 100 mL of the mobile phase and use this solution as the test solution. Perform the test immediately after the preparation of the test solution with 20 μL of the test solution as directed under Liquid Chromatography according to the following operating conditions. Determine each peak area by the automatic integration method and calculate the amount of each peak area by the area percentage method: the amount of epiminocycline is not more than 1.2 %, the amount of each peak other than minocycline and epiminocycline is not more than 1.0 %, and the total area of the peaks other than minocycline and epiminocycline is not more than 2.0 %.

Operating conditions

Detector, column, column temperature and mobile phase: Proceed as directed in the operating conditions in the Assay.

Flow rate: Adjust the flow rate so that the retention time of minocycline is about 12 minutes. The retention time of epiminocycline is about 10 minutes under this condition.

System suitability

System performance: Proceed as directed under the system suitability in the Assay.

Test for required detectability: Pipet 2 mL of the test solution, add the mobile phase to make exactly 100 mL and use this solution as the system suitability solution. Pipet 5 mL of the system suitability solution and add the mobile phase to make exactly 100 mL. Confirm that the peak area of minocycline obtained from 20 μL of this solution is equivalent to 3.5 % to 6.5 % of that from 20 μL of the minocycline suitability solution.

System repeatability: When the test is repeated 6 times with 20 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the peak area of minocycline is not more than 2.0 %.

Time span of measurement: About 2.5 times as long as the retention time of minocycline beginning after the solvent peak.

Loss on Drying Not more than 10.0 % (1.0 g, in vacuum, 100 °C, 5 hours)

Water 4.3 ~ 8.0 % (0.3 g, volumetric titration, direct titration)

Residue on Ignition Not more than 0.5 % (1.0 g)

Sterility Test It meets the requirement, when Minocycline Hydrochloride is used in a sterile preparation.

Bacterial Endotoxins Less than 1.25 EU/mg (potency), when Minocycline Hydrochloride is used in a sterile preparation.

Histamine It meets the requirement, when Minocycline Hydrochloride is used in a sterile preparation. Weigh appropriate amount of Minocycline Hydrochloride, dissolve in Isotonic Sodium Chloride Injection, make the solution so that each 1 mL contains 5.0 mg (potency), and use the solution as the test solution. Use 0.6 mL of the solution for the test.

Assay Weigh accurately about 50 mg (potency) each of Minocycline Hydrochloride and Minocycline Hydrochloride RS, dissolve in water to make exactly 100 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 20 μL each of these solutions as directed under Liquid Chromatography according to the following operating conditions and determine the peak areas, A_T and A_S, of minocycline.

$$\text{Amount [μg (potency)] of minocycline } (C_{23}H_{27}N_3O_7) = \text{Amount [μg (potency) of Minocycline Hydrochloride RS]} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Adjust the pH of a mixture of ammonium oxalate monohydrate solution (7 in 250), *N,N*-dimethylformamide and 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS (11:5:4) to 6.5 with tetrabutylammonium hydroxide TS.

Flow rate: Adjust the flow rate so that the retention time of minocycline is about 12 minutes.

System suitability

System performance: Dissolve 50 mg of Minocycline Hydrochloride in 25 mL of water. Heat 5 mL of this solution in a water-bath for 60 minutes and add water to make 25 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, epiminocycline and minocycline are eluted in this order with the resolution between their peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard

deviation of the peak area of minocycline is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Minocycline Hydrochloride Capsules

Minocycline Hydrochloride Capsules contain not less than 90.0 % and not more than 120.0 % of the labeled amount of minocycline ($C_{23}H_{27}N_3O_7$: 457.48).

Method of Preparation Prepare as directed under Capsules, with Minocycline Hydrochloride.

Identification Weigh an amount of Minocycline Hydrochloride Capsules, equivalent to 50 mg (potency) of minocycline hydrochloride according to the labeled amount, and about 50 mg (potency) of Minocycline Hydrochloride RS, dissolve each in 20 mL of methanol and use these solutions as the test solution and the standard solution, respectively. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, methanol and 10 % citric acid solution (4:1:2) to a distance of about 10 cm. Examine under ultraviolet light (main wavelength: 254 nm): the spots from the test solution and the standard solution show the same R_f value.

Loss on Drying Not more than 12.0 % (1.0 g, not exceeding 0.7 kPa, 100 °C, 5 hours)

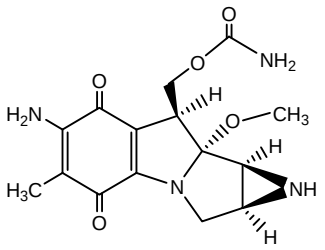
Disintegration Test It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Perform the test as directed in the Assay under Minocycline Hydrochloride. Weigh accurately the contents of not less than 20 Minocycline Hydrochlrodie Capsules and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg (potency) according to the labeled potency, dissolve in water to make exactly 50 mL and use this solution s the test solution. Weigh accurately about 50 mg (potency) of Minocycline Hydrochloride RS, dissolve in water to make exactly 50 mL and use this solution as the standard solution.

Containers and Storage ***Containers*—**Tight containers.

Mitomycin C



$C_{15}H_{18}N_4O_5$: 334.33

(1a*S*,8*S*,8a*R*,8b*S*)-6-Amino-4,7-dioxo-8a-methoxy-5-methyl-1,1a,2,8,8a,8bhexahydroazirino[2′,3′:3,4] pyrrolo[1,2-a]indol-8-ylmethyl carbamate [*50-07-7*]

Mitomycin C is a substance having antitumor activity produced by the growth of *Streptomyces caespitosus*. Mitomycin C contains not less than 970 μg (potency) and not more than 1030 μg (potency) per mg of mitomycin C ($C_{15}H_{18}N_4O_5$: 334.33), calculated on the dried basis.

Description Mitomycin C appears as blue-purple crystals or crystalline powder.
Mitomycin C is freely soluble in *N,N*-dimethylacetamide, slightly soluble in water or in methanol, and very slightly soluble in ethanol (99.5).

Identification (1) Determine the absorption spectra of solutions of Mitomycin C and Mitomycin C RS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Mitomycin C and Mitomycin C RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Crystallinity Test It meets the requirement.

pH The pH of a solution obtained by dissolving 0.1 g of Mitomycin C in 10 mL of water is between 5.5 and 7.5.

Absorbance $E_{1cm}^{1\%}$ (362 nm): 650 ~ 750 (1.0 mg, water, 100 mL).

Purity ***Related substances*—**Conduct this procedure rapidly after the test and standard solutions are prepared. Dissolve 50 mg of Mitomycin C in 10 mL of methanol, and use this solution as the test solution. Pipet 1 mL of the test solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following

conditions, and determine each peak area by the automatic integration method: each area of the peak other than mitomycin C obtained from the test solution is not larger than the peak area of mitomycin C from the standard solution, and the total area of the peaks other than mitomycin C from the test solution is not larger than 3 times the peak area of mitomycin C from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30 °C

Mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: To 20 mL of 0.5 mol/L ammonium acetate TS add water to make 1000 mL. To 800 mL of this solution add 200 mL of methanol.

Mobile phase B: To 20 mL of 0.5 mol/L ammonium acetate TS add water to make 1000 mL. To this solution add 1000 mL of methanol.

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-10	100	0
10-30	100→0	0→100
30-45	0	100

Flow rate: 1.0 mL/minute

System suitability

Test for required detectability: Pipet 10 mL of the standard solution, and add methanol to make exactly 100 mL. Confirm that the peak area of mitomycin C obtained from 10 μL of this solution is equivalent to 7 to 13 % of that from 10 μL of the standard solution.

System performance: Dissolve 25 mg of Mitomycin C and 40 mg of 3-ethoxy-4-hydrobenzaldehyde in 50 mL of methanol. When the procedure is run with 10 μL of this solution under the above operating conditions, mitomycin C and 3-ethoxy-4-hydrobenzaldehyde are eluted in this order with the resolution between these peaks being not less than 15.

System repeatability: When the test is repeated 3 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mitomycin C is not more than 3.0 %.

Time span of measurement: About 2 times as long as the retention time of mitomycin C beginning after the solvent peak.

Loss on Drying Not more than 1.0 % (0.1 g, reduced pressure not exceeding 0.67 kPa, 60 °C, 3 hours)

Sterility Test It meets the requirement, when Mitomycin C is used in a sterile preparation.

Bacterial Endotoxins Less than 50 EU/mg (potency) of mitomycin C, when Mitomycin C is used in a sterile preparation.

Assay Weigh accurately about 25 mg (potency) of Mitomycin C and Mitomycin C RS, dissolve each in *N,N*-dimethylacetamide to make exactly 50 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions and determine the peak areas, A_T and A_S, of mitomycin C.

$$\text{Amount [μg (potency)] of mitomycin C } (C_{15}H_{18}N_4O_5) = \text{Amount [μg (potency)] of mitomycin C RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 365 nm)

Column: A stainless steel column, about 4 mm in internal diameter and about 30 cm in length, packed with phenylated silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: To 40 mL of 0.5 mol/L ammonium acetate TS add 5 mL of diluted acetic acid (100) (1 in 20) and water to make 1000 mL. To 600 mL of this solution add 200 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of mitomycin C is about 7 minutes.

System suitability

System performance: Dissolve about 25 mg of Mitomycin C RS and 0.375 g of 3-ethoxy-4-hydroxybenzaldehyde in 50 mL of *N,N*-dimethylacetamide. When the procedure is run with 10 μL of this solution under the above operating conditions, mitomycin C and 3-ethoxy-4-hydroxybenzaldehyde are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mitomycin C is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Mitomycin C for Injection

Mitomycin C for Injection is a preparation for injection, which is dissolved before use. Mitomycin C for Injection contains not less than 90.0 % and not more than 110.0 % of the labeled amount of mitomycin C ($C_{15}H_{18}N_4O_5$: 334.33).

Method of Preparation Prepare as directed under Injections, with Mitomycin C.

Description Mitomycin C for Injection appears as blue-purple powder.

Identification Dissolve an amount of Mitomycin C for Injection, equivalent to 2 mg (potency) of Mitomycin C according to the labeled amount, in 200 mL of water, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 216 nm and 220 nm, and between 362 nm and 366 nm.

pH The pH of a solution, prepared by dissolving 0.25 g (potency) of Mitomycin C for Injection in 20 mL of water, is between 5.5 and 8.5.

Loss on Drying Not more than 1.0 % (0.4 g, in vacuum not exceeding 0.67 kPa, P_2O_5, 60 °C, 3 hours)

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 10 EU/mg (potency) of mitomycin C.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units Perform the test according to the following method: it meets the requirement of the Content Uniformity Test. To 1 container of Mitomycin C for Injection add exactly *V* mL of *N,N*-dimethyl-acetamide so that each mL contains about 0.5 mg (potency) of Mitomycin C, shake, centrifuge, and use the clear supernatant liquid as the test solution. Separately, weigh accurately about 25 mg (potency) of Mitomycin C RS, add *N,N*-dimethyl-acetamide to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Mitomycin C.

Amount [mg (potency)] of mitomycin C ($C_{15}H_{18}N_4O_5$)
= Amount [mg (potency)] of Mitomycin C RS

$$\times \frac{A_T}{A_S} \times \frac{V}{50}$$

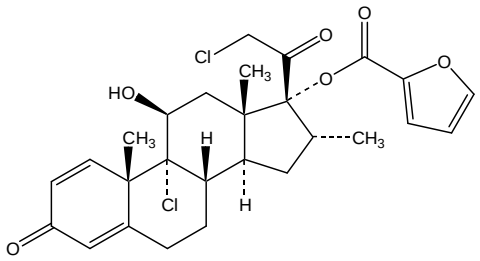
Assay Perform the test as directed in the Assay under Mitomycin C. Weigh accurately the mass of the contents of not less than 10 containers of Mitomycin C for Injection. Weigh accurately an amount of the contents, equivalent to about 10 mg (potency) of Mitomycin C according to the labeled potency, add exactly 20 mL of *N,N*-dimethylacetamide, shake, centrifuge, and use the clear supernatant liquid as the test solution. Separately, weigh accurately an amount of Mitomycin C RS, equivalent to about 25 mg (potency), dissolve in *N,N*-dimethyl-acetamide to make exactly 50 mL, and use this solution as the standard solution.

Amount [mg (potency)] of mitomycin C ($C_{15}H_{18}N_4O_5$)
= Amount [mg (potency)] of Mitomycin C RS

$$\times \frac{A_T}{A_S} \times \frac{2}{5}$$

Containers and Storage ***Containers***—Hermetic containers.

Mometasone Furoate



$C_{27}H_{30}Cl_2O_6$: 521.43

(8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*R*,17*R*)-9-Chloro-17-(2-chloroacetyl)-11-hydroxy-10,13,16-trimethyl-3-oxo-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[a]phenanthren-17-yl furan-2-carboxylate [*83919-23-7*]

Mometasone Furoate contains not less than 97.0 % and not more than 102.0 % of mometasone furoate ($C_{27}H_{30}Cl_2O_6$), calculated on the dried basis.

Description Mometasone Furoate appears as white powder.
Mometasone Furoate is freely soluble in acetone or in dichloromethane, slightly soluble in ethanol (95), and practically insoluble in water.

Identification (1) Determine the infrared spectra of Mometasone Furoate and Mometasone Furoate RS as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) When the procedure in the Assay is performed, the retention time of the major peak in the chromatogram obtained from the test solution corresponds to

that of the standard solution.

Specific Optical Rotation $[\alpha]_D^{20}$: +50° ~ +55° (50 mg after drying, ethanol (95), 10 mL, 100 mm).

Purity (1) ***Heavy metals*—**Proceed with 0.1 g of Mometasone Furoate according to Method 2 and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

(2) ***Related substances*—**Weigh accurately about 0.1 g of Mometasone Furoate, dissolve in dichloromethane to make exactly 10 mL, and use this solution as the test solution. Separately, dissolve an accurately weighed 0.1 g of Mometasone Furoate RS in dichloromethane to make exactly 10 mL. Dilute portions of this solution with dichloromethane to obtain standard solutions (1), (2), (3), (4), and (5) containing 0.5, 0.2, 0.1, 0.02, and 0.01 mg per mL, respectively. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 40 μL each of the test solution and the standard solutions (1), (2), (3), (4), and (5) on a plate of silica gel for thin-layer chromatography. Develop the plate in a chamber, previously equilibrated with a solvent system consisting of a mixture of chloroform and ethyl acetate (3 : 1), to a distance of about 15 cm. Remove the plate from the developing chamber, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm). Compare the intensities of any secondary spots observed in the chromatogram of the test solution with those of the principal spots in the chromatogram of the standard solutions: no secondary spot from the chromatogram of the test solution is more intense than the principal spot obtained from the standard solution (3) (not more than 0.1 %), and the sum of the intensities of the secondary spots from the test solution is not more than 2.0 %.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1g).

Assay Weigh accurately about 50 mg each of Mometasone Furoate and Mometasone Furoate RS, and dissolve in methanol to make exactly 50 mL. Pipet 5.0 mL each of these solutions, and dilute with a mixture of methanol, water, and acetic acid (31) (65 : 35 : 0.2) to make exactly 50 mL. Pipet 10 mL of these solutions and 10.0 mL of the internal standard solution into a 50-mL volumetric flask, and dilute with a mixture of methanol, water, and acetic acid (31) (65 : 35 : 0.2) to make exactly 50 mL. Use these solutions as the test solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of mometasone furoate to that of the internal standard, respectively.

$$\text{Amount (mg) of mometasone furoate } (C_{27}H_{30}Cl_2O_6) = \text{Amount (mg) of Mometasone Furoate RS} \times \frac{Q_T}{Q_S}$$

Internal standard—Weigh 40 mg of beclomethasone dipropionate, and dissolve in a mixture of methanol, water, and acetic acid (31) (65:35:0.2) to make 100 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4.6 mm internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm particle diameter).

Mobile phase: A mixture of methanol and water (65:35).

Flow rate: 1.7 mL/min.

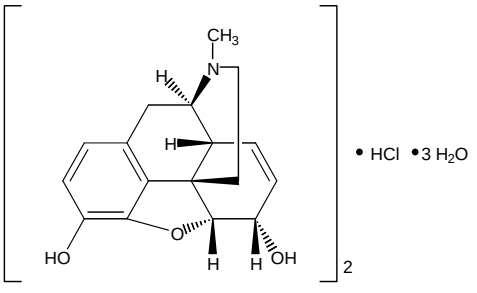
System suitability

System performance: When the test is performed with 20 μL of the standard solution according to the above operating conditions, the relative retention times are about 1.6 for beclomethasone dipropionate and 1.0 for mometasone furoate, the resolution between these peaks is not less than 4.0, and the symmetry factor for the mometasone furoate peak is not more than 1.8.

System repeatability: When the test is repeated 5 times with 20 μL each of the standard solution according to the above conditions, the relative standard deviation of the peak area of mometasone furoate is not more than 2.0 %.

Containers and Storage ***Containers*—**Well-closed containers.

Morphine Hydrochloride Hydrate



Morphine Hydrochloride

$C_{17}H_{19}NO_3 \cdot HCl \cdot 3H_2O$: 375.84

(4*R*,4*aR*,7*S*,7*aR*,12*bS*)-3-Methyl-2,3,4,4a,7,7a-hexahydro-1*H*-4,12-methanobenzofuro[3,2-e]-isoquinoline-7,9-diol trihydrate hydrochloride [*6055-06-7*]

Morphine Hydrochloride Hydrate contains not less than 98.0 % and not more than 102.0 % of morphine hydrochloride ($C_{17}H_{19}NO_3 \cdot HCl$: 321.80), calculated on the anhydrous basis.

Description Morphine Hydrochloride Hydrate appears as white crystals or crystalline powder.
Morphine Hydrochloride Hydrate is freely soluble in formic acid, soluble in water, sparingly soluble in methanol, slightly soluble in ethanol (95) and practically insoluble in ether.
Morphine Hydrochloride Hydrate gradually becomes yellow-brown by light.

Identification (1) Determine the absorption spectra of the aqueous solutions of Morphine Hydrochloride Hydrate and Morphine Hydrchloride Hydrate RS (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectra of the solutions of Morphine Hydrochloride Hydrate and Morphine Hydrochloride Hydrate RS in dilute sodium hydroxide TS (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Morphine Hydrochloride Hydrate and Morphine Hydrochloride Hydrate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Morphine Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests (2) for Chloride.

Specific Optical Rotation $[\alpha]_D^{20}$: -111 ~ -116° (0.5 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.1 g of Morphine Hydrochloride Hydrate in water is between 4.0 and 6.0.

Purity (1) ***Clarity of solution***—Dissolve 0.40 g of Morphine Hydrochloride Hydrate in 10 mL of water: the solution is clear. Determine the absorbance at 420 nm of this solution as directed under Ultraviolet-visible Spectrophotometry: not more than 0.12.

(2) ***Sulfate***—Dissolve 0.20 g of Morphine Hydrochloride Hydrate in 5 mL of water and add 2 to 3 drops of barium chloride TS: no turbidity is produced.

(3) ***Meconic acid***—Dissolve 0.20 g of Morphine Hydrochloride Hydrate in 5 mL of water and add 5 mL of dilute hydrochloric acid and 2 drops of iron (III) chloride TS: no red color is observed.

(4) ***Related substances***—Dissolve 0.1 g of Morphine Hydrochloride Hydrate in 10 mL of diluted ethanol (99.5) (1 in 2) and use this solution as the test solution. Pipet 1.0 mL of the test solution, add diluted ethanol (99.5) (1 in 2) to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), toluene, acetone and ammonia solution (28) (14 : 14 : 7 : 1) to a distance of about 15 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Water 13.0 ~ 15.0 % (0.1 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (0.5 g).

Assay Weigh accurately about 0.5 g of Morphine Hydrochloride Hydrate, dissolve in 3.0 mL of formic acid, add 80 mL of a mixture of acetone and acetic acid (100) (1 : 1) and 3.0 mL of bismuth nitrate TS, mix and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make a necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.180 mg of $C_{17}H_{19}NO_3 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.
Storage—Light-resistant.

Morphine Hydrochloride Injection

Morphine Hydrochloride Injection is an aqueous solution for injection. Morphine Hydrochloride Injection contains not less than 93.0 % and not more than 107.0 % of the labeled amount of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3 \cdot HCl \cdot 3H_2O$: 375.85).

Method of Preparation Prepare as directed under Injections, with Morphine Hydrochloride Hydrate.

Description Morphine Hydrochloride Injection is a clear and colorless to pale yellow-brown liquid.
Morphine Hydrochloride Injection gradually becomes yellow-brown by light.
pH—2.5 ~ 5.0.

Identification Take a volume of Morphine Hydrochloride Injection, equivalent to 40 mg of Morphine Hydrochloride Hydrate according to the labeled amount, add water to make 20mL and use this solution

as the test solution. Add water to 5 mL of the test solution to make 100 mL and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 283 nm and 287 nm. Add diluted sodium hydroxide TS to 5 mL of the test solution to make 100 mL and determine the absorption spectrum: it exhibits a maxiumum between 296 nm and 300 mn.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 1.5 EU/mg of morphine hydrochloride.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Iinjection It meets the requirement.

Determination of Volume of Injection in Container It meets the requirement.

Assay Take accurately a volume of Morphine Hydrochloride Injection, equivalent to about 20 mg of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3 \cdot HCl \cdot 3H_2O$) according to the labeled amount, add the mobile phase to make exactly 100 mL and use this solution as the test solution. Separatly, weigh accurately about 20 mg of Morphine Hydrochloride Hydrate RS, dissolve in the mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform the test with 25 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the peak areas, A_T and A_S, of the test solution and the standard solution.

Amount (mg) of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3 \cdot HCl \cdot 3H_2O$)
= Amount (mg) of Morphine Hydrochloride Hydrate RS $\times \dfrac{A_T}{A_S}$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 284 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Dissolve 0.73 g of sodium 1-heptane-sulfonate in 720 mL of water, add 280 mL of methanol and 10 mL of acetic acid (100) and filter.

Flow rate: 1.5 mL/minute

System suitability

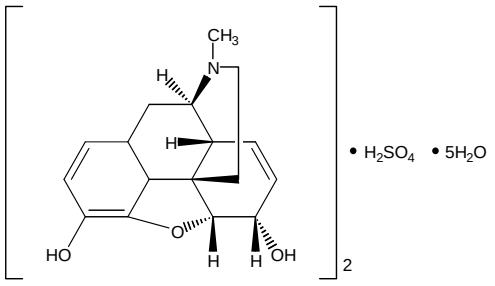
System performance: When the procedure is run with 25 μL of the standard solution under the above operating conditions, the symmetry factor is not more than 2.0.

System repeatability: When the test is repeated 6 times with 25 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area is not more than 2.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Storage—Light-resistant.

Morphine Sulfate Hydrate



Morphine Sulfate

$(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$: 758.83

(4*R*,4*aR*,7*S*,7*aR*,12*bS*)-3-Methyl-2,3,4,4a,7,7a-hexahydro-1*H*-4,12-methanobenzofuro[3,2-e]-isoquinoline-7,9-diol; sulfuric acid; pentahydrate [*6211-15-0*]

Morphine Sulfate Hydrate contains not less than 98.0 % and not more than 102.0 % of morphine sulfate [$(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4$: 668.75], calculated on the anhydrous basis.

Description Morphine Sulfate Hydrate appears as lustrous crystals like hair or silk, columnar crystals or white crystalline powder, is orderless and loses its water of hydration gradually on stand in the air.

Morphine Sulfate Hydrate is very soluble in hot water, soluble in water, sparingly soluble in ethanol (95) or in warm ethanol and practically insoluble in chloroform or in ether.

Morphine Sulfate is colored by light.

Identification (1) Take 1 mg of Morphine Sulfate Hydrate in a porcelain crucible or small dish, add 0.5 mL of sulfuric acid containing 1 drop of formaldehyde TS per mL: a deep purple color is observed at once and quickly changes to deep blue-purple (distinction from codeine, which gives at once a deep purple-blue color and from hydromorphone, which gives at first a yellow-brown color, changing to pink-purple and then to red-purple).

(2) Dissolve 5 mg of Morphine Sulfate Hydrate in 5 mL of sulfuric acid, add 1 drop of iron (III) chloride TS, mix and heat in boiling water for 2 minutes: a blue color is observed and when 1 drop of nitric acid is add-

ed, it changes to dark red-brown (codeine and ethylmorphine give the same color reactions, but hydromorphone and papaverine do not produce this color change).

(3) Determine the infrared spectra of Morphine Sulfate Hydrate and Morphine Sulfate Hydrate RS, previously dried at 145 ºC for 1 hour, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(4) An aqueous solution of Morphine Sulfate Hydrate (1 in 50) responds to the Qualitative Tests for sulfate.

Specific Optical Rotation $[\alpha]_D^{25}$: -107 ~ -109.5º (0.1 g, calculated on the dried basis, water, 10 mL, 100 mm).

Purity (1) ***Acid***—Dissolve 0.5 g of Morphine Sulfate Hydrate in 15 mL of water, add 1 drop of methyl red TS and titrate with 0.02 mol/L sodium hydroxide until a yellow color is observed: not more than 0.50 mL is required.

(2) ***Chloride***—Take 10 mL of a aqueous solution (1 in 100) of Morphine Sulfate Hydrate, add 1 mL of 2 mol/L nitric acid and 1 mL of silver nitrate TS: no precipitate or turbidity is produced immediately.

(3) ***Ammonium salts***—Heat 0.2 g of Morphine Sulfate Hydrate with 5 mL of 1 mol/L sodium hydroxide TS in a water-bath for 1 minute: no odor of ammonia is perceptible.

(4) ***Related substances***—Dissolve 1.0 g of Morphine Sulfate Hydrate in 10 mL of 1 mol/L sodium hydroxide in a separatory funnel and shake the solution with three successive portions of 15 mL, 10 mL and 10 mL of chloroform, passing the chloroform solutions through a small filter previously moistened with chloroform. Shake the combined chloroform solutions with 5 mL of water, separate the chloroform layer and evaporate on a water-bath to dryness. To the residue, add 10.0 mL of 0.01 mol/L sulfuric acid VS and heat gently until dissolved. Cool, add 2 drops of methyl red TS and titrate the excess acid with 0.02 mol/L sodium hydroxide VS: not less than 7.5 mL is required (1.5 %).

Water 10.4 ~ 13.4 % (0.2 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (0.5 g).

Assay Weigh accurately about 24 mg of Morphine Sulfate Hydrate, add the mobile phase to make exactly 100 mL and use this solution as the test solution. Separately, weigh accurately a portion of Morphine Sulfate Hydrate RS, previously measured the content of water, dissolve in mobile phase to obtain a solution having a known concentration of about 0.24 mg per mL and use this solution as the standard solution. Prepare the standard solution when used. Perform the test with 25 µL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and determine the peak areas, A_T and A_S, of morphine for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of morphine sulfate } [(C_{17}H_{19}NO_3)_2{\cdot}H_2SO_4] = 100 \times C \times \frac{A_T}{A_S}$$

C: Concentrtion (mg/mL) of anhydrous morphine sulfate in the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 284 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 30 cm in length, having octadecylsilanized silica gel for liquid chromatography (3 µm to 10 µm in particle diameter).

Mobile phase: Dissolve 0.73 g of sodium 1-heptanesulfonate in 720 mL of water, add 280 mL of methanol and 10 mL of acetic acid (100), mix and filter.

Flow rate: 1.5 mL/minute.

System suitability

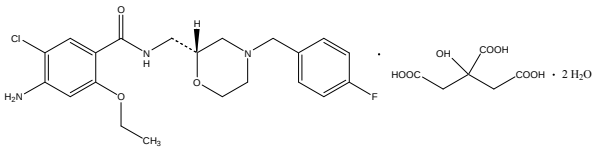
System performance: When the procedure is run with 25 µL of the standard solution according to the above operating conditions, the symmetry factor is not more than 2.0.

System repeatability: When the test is repeated 6 times with 25 µL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of morphine is not more than 2.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Mosapride Citrate Hydrate



and enantiomer

$C_{21}H_{25}ClFN_3O_3{\cdot}C_6H8O_7{\cdot}2H_2O$: 650.05

4-Amino-5-chloro-2-ethoxy-*N*-{[(2*RS*)-4-(4-fluorobenzyl)morpholin-2-yl]methyl}-benzamide monocitrate dihydrate [*636582-62-2*]

Mosapride Citrate Hydrate contains not less than 98.5 % and not more than 101.0 % of mosapride citrate ($C_{21}H_{25}ClFN_3O_3{\cdot}C_6H_8O_7$: 614.02), calculated on the anhydrous basis.

Description Mosapride Citrate Hydrate appears as white to pale yellow crystalline powder.
Mosapride Citrate Hydrate is freely soluble in *N,N*-dimethylformamide or in acetic acid (100), sparingly soluble in methanol, slightly soluble in ethanol (99.5) and practically insoluble in water.
A solution of Mosapride Citrate Hydrate in *N,N*-dimethylformamide (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectrum of the solution of Mosapride Citrate Hydrate in methanol (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectrum of Mosapride Citrate Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Mosapride Citrate Hydrate in *N,N*-dimethylformamide (1 in 10) responds to the Qualitative Tests (1) for citrate.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Mosapride Citrate Hydrate in a platinum crucible according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Dissolve 0.10 g of Mosapride Citrate Hydrate in 50 mL of methanol and use this solution as the test solution. Pipet 1 mL of this solution and add methanol to make exactly 50 mL. Pipet 1 mL of this solution, add methanol to make exactly 20 mL and use this solution as the standard solution. Perform the test with exactly 5 μL each of the test solution and the standard solution as directed under Liquid Chromatography under the following operating conditions. Determine each peak area in each solution by the automatic integration method: the area of the peak having the relative retention time of about 0.47 with respect to mosapride from the test solution is not larger than 3 times the peak area of mosapride from the standard solution, and the area of each peak other than the peak of mosapride and other than the peak mentioned above is not larger than the peak area of mosapride from the standard solution. The total area of the peaks other than the peak of mosapride from the test solution is not larger than 5 times the peak area of mosapride from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 274 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: Control the step or concentration gradient by mixing mobile phases A and B as directed in the following table.

Mobile phase A: Dissolve 8.82 g of trisodium citrate dihydrate in 800 m L of water, adjust the pH to 4.0 with dilute hydrochloric acid and add water to make 1000 mL.

Mobile phase B: Acetonitrile

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-35	80→45	20→55

Flow rate: 1.0 mL/minute

System suitability

Test for required detectability: Pipet 4 mL of the standard solution and add methanol to make exactly 20 mL. Confirm that the peak area of mosapride obtained from 5 μL of this solution is equivalent to 15 % to 25 % of the peak area of mosapride from the standard solution.

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of mosapride are not less than 40000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mosapride is not more than 5.0 %.

Time span of measurement: 35 minutes beginning after the solvent peak

Water 5.0 ~ 6.5 % (0.5 g, volumetric titration, back titration)

Residue on Ignition Not more than 0.1 % (1 g, platinum crucible)

Assay Weigh accurately about 0.5 g of Mosapride Citrate Hydrate, dissolve in 70 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 61.40 mg of $C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7$

Containers and Storage ***Containers***—Well-closed containers.

Mosapride Citrate Tablets

Mosapride Citrate Tablets contain not less than 95.0 % and not more than 105.0 % of the labeled amount of mosapride citrate ($C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7$: 614.02).

Method of Preparation Prepare as directed under Tablets, with Mosapride Citrate Hydrate.

Identification (1) Weigh a portion of powdered Mosapride Citrate Tablets, equivalent to 10 mg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7$), add 10 mL of dilute acetic acid, shake for 10 minutes and filter. To 5 mL of the filtrate, add 0.3 mL of Dragendorff's TS: an orange precipitate is produced.

(2) Determine the absorption spectrum of the test solution obtained from the Assay as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 271 nm and 275 nm and between 306 nm and 310 nm.

Purity ***Related substances***—Powder not less than 20 Mosapride Citrate Tablets. Weigh a portion of the powder, equivalent to 10 mg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7$) according to the labeled amount, and moisten with 1 mL of water. Add 9 mL of methanol, shake for 20 minutes, centrifuge and use the clear supernatant liquid as the test solution. Pipet 1 mL of this solution and add methanol to make exactly 20 mL. To 2 mL of this solution, add methanol to make exactly 20 mL and use this solution as the standard solution. Perform the test with 10 μL of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine each peak area of each solution by the automatic integration method: the area of the peaks having the relative retention times of about 0.60 and about 0.85 with respect to mosapride from the test solution is not larger than the peak area of mosapride from the standard solution, and the area of each peak other than the peak of mosapride and other than those mentioned above from the test solution is not larger than 2/5 times the peak area of mosapride from the standard solution. The total area of peaks other than mosapride from the test solution is not larger than 2 times the peak area of mosapride from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase A, mobile phase B and flow rate: Proceed as directed in the operating conditions in the Purity (2) under Mosapride Citrate Hydrate.

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as directed in the following table.

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-40	85→45	15→55

System suitability

Test for required detectability: Pipet 1 mL of the standard solution and add methanol to make exactly 25 mL. Confirm that the peak area of mosapride obtained from 10 μL of this solution is equivalent to 3.0 % to 5.0 % of the peak area of mosapride from the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of mosapride are not less than 40000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mosapride is not more than 3.0 %.

Time span of measurement: 40 minutes beginning after the solvent peak.

Dissolution Test Perform the test with 1 tablet of Mosapride Citrate Tablets at 50 revolutions per minute as directed under Method 2 under Dissolution Test, using 900 mL of the second solution as the dissolution solution. Take not less than 20 mL of the dissolved solution after 45 minutes from the start of the test and filter through a membrane filter with pore size of not more than 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution solution to make exactly V' mL so that each mL contains about 2.8 μg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7$) and use this solution as the test solution. Separately, weigh accurately about 30 mg of Mosapride Citrate RS (separately, determine the water in the same manner as Mosapride Citrate Hydrate) and dissolve in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 200 mL and use this solution as the standard solution. Perform the test with exactly 50 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the peak areas, A_T and A_S, of mosapride in each solution. The dissolution rate of Mosapride Citrate Tablets in 45 minutes is not less than 80 %.

Dissolution rate (%) with respect to the labeled amount of mosapride citrate ($C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7$)

$$= W_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 9$$

W_S: Amount (mg) of Mosapride Citrate RS, calculated on the anhydrous basis

C: Labeled amount (mg) of mosapride citrate ($C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7$) in 1 tablet

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 274 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: Dissolve 8.82 g of trisodium citrate dihydrate in 800 mL of water, adjust the pH to 3.3 with dilute hydrochloric acid and add water to make 1000 mL. To 240 mL of this solution, add 90 mL of methanol and 70 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of mosapride is about 9 minutes.

System suitability

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of mosapride are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL each of the standard solution, the relative standard deviation of the peak area of mosapride is not more than 2.0 %.

Uniformity of Dosage Units It meets the requirement.

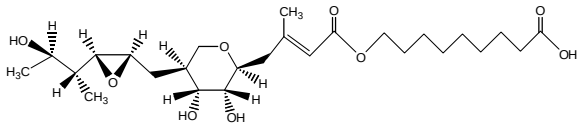
Assay Weigh accurately and powder not less than 20 Mosapride Citrate Tablets. Weigh accurately a portion of the powder, equivalent to about 10 mg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7$), and moisten with 2 mL of water. Add 70 mL of methanol, shake for 20 minutes, add methanol to make exactly 100 mL and centrifuge. Pipet 10 mL of the clear supernatant liquid, add methanol to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately about 53 mg of Mosapride Citrate RS (separately, determine the water in the same manner as Mosapride Citrate Tablets) and dissolve in methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 50 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 273 nm of the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of mosapride citrate } (C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7) = W_S \times \frac{A_T}{A_S} \times \frac{1}{5}$$

W_S: Amount (mg) of Mosapride Citrate RS, calculated on the dried basis

Containers and Storage ***Containers***—Tight containers.

Mupirocin



$C_{26}H_{44}O_9$: 500.62

9-[(*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-Dihydroxy-5-[[(2*S*,3*S*)-3-[(2*S*,3*S*)-3-hydroxybutan-2-yl]-oxiran-2-yl]methyl]oxan-2-yl]-3-methylbut-2-enoyl]oxynonanoic acid [*12650-69-0*]

Mupirocin is a substance having antibacterial activity produced by the growth of *Peudomonas fluorescens*. Mupirocin contains not less than 920 μg (potency) and not more than 1020 μg (potency) per mg of mupirocin ($C_{26}H_{44}O_9$: 500.62), calculated on the anhydrous basis.

Description Mupirocin appears as white powder. Mupirocin is freely soluble in methanol, in ethanol (95), in acetone or in chloroform, slightly soluble in ether, and very slightly soluble in water.

Identification Determine the absorption spectra of Mupirocin and Mupirocin RS as directed in the paste method under Infrared Spectrophotometry, both spectra exhibit similar intensities of absorption at the same wavenumvers.

pH The pH of a saturated solution of Mupirocin is between 3.5 and 4.5.

Purity ***Total related substances***—Weigh accurately about 0.1 g (potency) of Mupirocin, dissolve in the mixture of 0.1 mol/L ammonium acetate buffer solution, pH 4.0 and methanol (1:1), make to exactly 10 mL and use this solution as the test solution A. Pipet 5 mL of the test solution A, add the mixture of 0.1 mol/L ammonium acetate buffer solution, pH 4.0 and methanol (1:1) to make exactly 200 mL and use this solution as the test solution B. Perform the test with 20 μL of the test solution B as directed under Liquid Chromatography according to the following operating conditions and obtain the peak areas of related substances except solvent, A_1 and A_2 (not more than 8.0 %).

$$\text{Total amount (\%) of related substances} = \frac{A_2}{A_1 + A_2} \times 100$$

A_1: Peak area of mupirocin / Amount of mupirocin (mg) in one mL of the test solution B

A_2: (Total peak areas of related substances – Total peak of solvent) / Amount of mupirocin (mg) in one mL of the test solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 240 nm)

Column: A stainless steel column, about 4.6 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (6 μm in particle diameter)

Mobile phase: The mixture of 0.1 mol/L ammonium acetate buffer solution, pH 5.7 and tetrahydrofuran (75:25).

Flow rate: 2.0 mL / minute

Water Not more than 1.0 % (1.0 g, volumetric titration, direct titration).

Assay Weigh accurately about 11 mg (potency) each of Mupirocin and Mupirocin Lithium RS, dissolve in 25 mL of acetonitrile, add phosphate buffer (pH 6.3) to make exactly 100 mL and use these solutions as the test solution and the standard solution. Perform the test with 20 μL each of these solutions as directed under Liquid Chromatography according to the following operating conditions, and determine the peak areas, A_T and A_S of mupirocin.

Amount [μg (potency)] of mupirocin ($C_{26}H_{44}O_9$)
= Amount [μg (potency)] of Mupirocin Lithium RS

$$\times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm)

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (1.5 μm to 10 μm in particle diameter).

Mobile phase: A mixture of phosphate buffer (pH 6.3) and acetonitrile (75:25)

Flow rate: 2.0 mL/minute.

System suitability

System performance: Pipet 10 mL of the standard solution, adjust the pH to 2.0 with 6 mol/L hydrochloric acid, allow to stand for 2 hours, adjust the pH to 6.3 ± 0.2 with 5 mol/L sodium hydroxide and use this solution as the system suitability solution. When the procedure is run with this solution under the above operating conditions, the relative retention times of the mupirocin acid hydrolysis product and mupirocin are 0.9 and 1.0, respectively, with the resolution between their peaks being not less than 2.0. When the procedure is run with the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of mupirocin is not less than 1500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 5 times with the standard solution under the above operating conditions, the relative standard deviation of the peak of mupirocin is not more than 2.0 %.

Phosphate buffer (pH 6.3)—Adjust the pH of 0.05 mol/L sodium dihydrogen phosphate monohydrate to 6.3 ± 0.2 with 10 mol/L sodium hydroxide.

Containers and Storage ***Containers***—Tight containers.

Mupirocin Calcium Cream

Mupirocin Calcium Cream contains not less than 90.0 % and not more than 120.0 % of the labeled amount of mupirocin ($C_{26}H_{44}O_9$: 500.62).

Method of Preparation Prepare as directed under Creams, with Mupirocin Calcium Hydrate.

Identification When proceed as directed in the Assay, the retention time of the principal peak from the test solution corresponds to that from the standard solution.

pH The pH of a solution obtained by dissolving an amount of Mupirocin Calcium Cream, equivalent to 0.1 g (potency) of mupirocin, in 10 mL of water is between 6.0 and 8.0.

Purity ***Related substances***—Perform the test as directed in the Assay. Weigh accurately an amount of Mupirocin Calcium Cream, equivalent to about 50 mg (potency), add 5 mL of tetrahydrofuran and mix well. Add 5 mL of 0.1 mol/L sodium acetate RS, centrifuge, filter the bottom layer and use the filtrate as the test solution. If it contains a preservative agent, prepare a standard solution of the preservative agent and check the location of the peak of the preservative agent in the test solution (not more than 8.5 %).

$$\text{Amount (\%) of related substances} = \frac{A_R}{A_T} \times 100$$

A_T: Total area of all peaks from the test solution excluding the peak of the preservative agent

A_R: Total peak area of all related substances

Assay Weigh accurately an amount of Mupirocin Calcium Cream, equivalent to about 20 mg (potency) according to the labeled potency, dissolve in 75 mL of a mixture of 0.5 mol/L sodium phosphate buffer (pH 6.3) and tetrahydrofuran (2:1), add 0.5 mol/L phosphate buffer (pH 6.3) to make exactly 200 mL and use this solution as the test solution. Separately, weigh accurately about 20 mg (potency) of Mupirocin Lithium RS, dissolve in 75 mL of a mixture of 0.5 mol/L sodium phosphate buffer (pH 6.3) and tetrahydrofuran (2:1), add 0.5 mol/L phosphate buffer (pH 6.3) to make exactly 200 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid

Chromatography and determine the peak areas, A_T and A_S, of mupirocin.

$$\text{Amount [μg (potency)] of mupirocin } (C_{26}H_{44}O_9) = \text{Amount [μg (potency)] of Mupirocin Lithium RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 240 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octylsilyl silica gel for liquid chromatography (10 μm in particle diameter).

Flow rate: 1.0 mL/minute

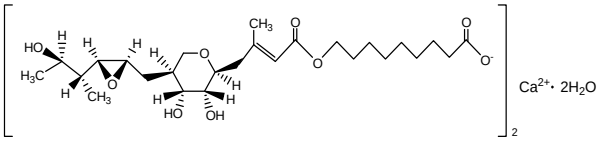
Mobile phase: Maintain solution A for 10 minutes, make 100 % solution B over 35 minutes in a linear gradient and maintain solution B for 20 minutes.

Solution A: A mixture of 0.1 mol/L ammonium acetate buffer (pH 5.7) and tetrahydrofuran (3:1)

Solution B: A mixture of 0.1 mol/L ammonium acetate buffer (pH 5.7) and tetrahydrofuran (7:2)

Containers and Storage ***Containers***—Tight containers.

Mupirocin Calcium Hydrate



$C_{52}H_{86}O_{18}Ca \cdot 2H_2O$: 1075.34

Calcium 9-[(*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-[[(2*S*,3*S*)-3-[(2*S*,3*S*)-3-hydroxybutan-2-yl]oxiran-2-yl]methyl]oxan-2-yl]-3-methylbut-2-enoyl]oxynonanoate dihydrate [*115074-43-6*]

Mupirocin Calcium Hydrate is the calcium salt of a substance having antibacterial activity produced by the growth of *Peudomonas fluorescens*.

Mupirocin Calcium Hydrate contains not less than 895 μg (potency) and not more than 970 μg (potency) per mg of mupirocin ($C_{26}H_{44}O_9$: 500.62), calculated on the anhydrous basis.

Description Mupirocin Calcium Hydrate appears as white powder and has a bitter taste.

Mupirocin Calcium Hydrate is freely soluble in methanol and slightly soluble in water or in ethanol (95).

Identification (1) To 1 mL of a solution of Mupirocin Calcium Hydrate in methanol (1 in 200), add 4 mL of hydroxylamine perchlorate-ethanol (99.5) TS and 1 mL of *N,N*-dicyclohexylcarbodiimide-ethanol (99.5) TS, shake well and allow to stand in lukewarm water for 20 minutes. After cooling, add 1 mL of iron (III) perchlorate-ethanol (99.5) TS and shake: a dark purple color develops.

(2) Determine the absorption spectrum of a solution of Mupirocin Calcium Hydrate (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 219 nm and 224 nm.

(3) Determine the infrared spectrum of Mupirocin Calcium Hydrate as directed in the paste method under Infrared Spectrophotometry: it iexhibits absorption at the wavenumbers of about 1708 cm^{-1}, 1648 cm^{-1}, 1558 cm^{-1}, 1231 cm^{-1}, 1151 cm^{-1} and 894 cm^{-1}.

(4) A solution of Mupirocin Calcium Hydrate (3 in 1000) responds to the Qualitative Tests (3) for calcium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: -16 ~ 20° (1 g calculated on the anhydrous basis, methanol, 20 mL, 100 mm)

Purity (1) ***Chloride***—Weigh accurately about 50 mg of Mupirocin Calcium Hydrate, dissolve in a mixture of 1 mL of 2 mol/L nitric acid and 15 mL of methanol, use this solution as the test solution and perform the test. Prepare the control solution by adding a mixture of 1 mL of 2 mol/L nitric acid and 15 mL of methanol to 0.7 mL of 0.02 mol/L hydrochloric acid (not more than 0.5 %).

(2) ***Related substances***—Dissolve about 50 mg of Mupirocin Calcium Hydrate in a mixture of 0.1 mol/L acetic acid-sodium acetate buffer (pH 4.0) and tetrahydrofuran solution (3 in 4) (1:1) to make 10 mL and use this solution as the test solution (1). Pipet 2 mL of this solution, add a mixture of 0.1 mol/L acetic acid-sodium acetate buffer (pH 4.0) and tetrahydrofuran solution (3 in 4) (1 : 1) to make exactly 100 mL and use this solution as the test solution (2). Preserve these test solutions at a temperature between 4 °C and 8 °C. Perform the test with exactly 20 μL each of the test solution (1) and the test solution (2) as directed under Liquid Chromatography according to the following operating conditions and determine the areas of each peak of the test solution (1) and the test solution (2) by the automatic integration method: the amount of the principal related substance having the relative retention time of about 0.7 with respect to mupirocin is not more than 4.0 %, and the total amount of related substances other than the peaks of the solvent and mupirocin is not more than 6.0 %.

$$\text{Amount (\%) of principal related substance} = \frac{A_i}{A + A_m} \times 100 \times \frac{P \times 100}{100 - \dfrac{A \times 100}{A + A_m}}$$

Total amount (%) of related substances

$$= \frac{A}{A + A_m} \times 100 \times \frac{P \times 100}{100 - \frac{A \times 100}{A + A_m}}$$

A: Total peak areas other than of the solvent and mupirocin obtained from the test solution (1)

A_i: Area of the peak having the relative retention time of about 0.7 with respect to mupirocin obtained from the test solution (1)

A_m: A value of 50 times the peak area of mupirocin obtained from the test solution (2)

P: Amount [mg (potency)] of mupirocin ($C_{26}H_{44}O_9$) per mg of Mupirocin Calcium Hydrate, obtained from the Assay

Operating conditions

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Pipet 1 mL of the test solution (2) and add a mixture of 0.1 mol/L acetic acid-sodium acetate buffer (pH 4.0) and tetrahydrofuran solution (3 in 4) (1 : 1) to make exactly 20 mL. Confirm that the peak area of mupirocin obtained from 20 μL of this solution is equivalent to 4 % to 6 % of the peak area from 20 μL of the test solution (2).

System repeatability: When the test is repeated 6 times with 20 μL each of the test solution (2) under the above operating conditions, the relative standard deviation of the peak area of mupirocin is not more than 2.0 %.

Time span of measurement: About 3 times as long as the retention time of mupirocin beginning after the solvent peak.

0.1 mol/L acetic acid-sodium acetate buffer (pH 4.0)—Dissolve 13.61 g of sodium acetate trihydrate in about 750 mL of water, adjust the pH to 4.0 with acetic acid (100) and add water to make 1000 mL.

Water 3.0 ~ 4.5 % (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately about 20 mg (potency) each of Mupirocin Calcium Hydrate and Mupirocin Lithium RS, dissolve in a mixture of acetic acid-sodium acetate buffer (pH 4.0) and tetrahydrofuran solution (3 in 4) (1 : 1) to make exactly 200 mL and use these solutions as the test solution and the standard solution. Preserve these solutions at a temperature between 4 °C and 8 °C. Perform the test with 20 μL each of these solutions as directed under Liquid Chromatography according to the following operating conditions and determine the peak areas, A_T and A_S, of mupirocin.

Amount [μg (potency)] of mupirocin ($C_{26}H_{44}O_9$)

= Amount [μg (potency)] of Mupirocin Lithium RS

$$\times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 240 nm)

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: Dissolve 7.71 g of ammonium acetate in 750 mL of water, adjust the pH to 5.7 with acetic acid (100) and add water to make 1000 mL. To 300 mL of this solution, add 100 mL of tetrahydrofuran.

Flow rate: Adjust the flow rate so that the retention time of mupirocin is about 12.5 minutes.

System suitability

System performance: Dissolve about 20 mg of Mupirocin Lithium RS and about 5 mg of ethyl paraoxybenzoate in a mixture of 0.1 mol/L acetic acid-sodium acetate buffer (pH 4.0) and tetrahydrofuran solution (3 in 4) (1:1) to make 200 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, mupirocin and ethyl paraoxybenzoate are eluted in this order with the resolution between their peaks being not less than 12.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mupirocin is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Mupirocin Ointment

Mupirocin Ointment contains not less than 90.0 % and not more than 120.0 % of the labeled amount of mupirocin ($C_{26}H_{44}O_9$: 500.62).

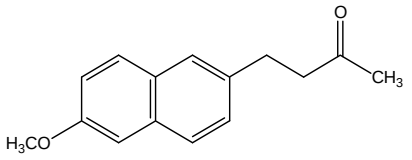
Method of Preparation Prepare as directed under Ointments, with Mupirocin.

Identification When proceed as directed in the Assay, the retention time of the principal peak from the test solution corresponds to that from the standard solution.

Assay Perform the test as directed in the Assay under Mupirocin. Weigh accurately an amount of Mupirocin Ointment, equivalent to about 10 mg (potency) according to the labeled potency, dissolve in 25 mL of acetonitrile, add phosphate buffer (pH 6.3) to make exactly 100 mL and use this solution as the test solution.

Containers and Storage ***Container***s—Tight containers.

Nabumetone



$C_{15}H_{16}O_2$: 228.29

4-(6-Methoxynaphthalen-2-yl)butan-2-one
[*42924-53-8*]

Nabumetone contains not less than 98.0 % and not more than 101.0 % of nabumetone ($C_{15}H_{16}O_2$), calculated on the anhydrous basis.

Description Nabumetone appears as white to pale yellow crystals or crystalline powder.
Nabumetone is freely soluble in acetone, sparingly soluble in methanol or in ethanol (95) and practically insoluble in water.

Melting point— 79 ~ 84 °C

Identification (1) Determine the infrared spectra of Nabumetone and Nabumetone RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) When proceed as directed in the Assay, the retention time of the principal peak from the test solution corresponds to that from the standard solution.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Glutamine according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Weigh accurately about 0.1 g of Nabumetone, add acetonitrile to make exactly 100 mL and use this solution as the test solution. Perform the test with 10 μL of the test solution as directed under Liquid Chromatography according to the following operating conditions and determine the area of the peaks from the test solution: the content of the related substance having the relative retention time of 2.7 is not more than 0.3 %, the content of any other related substance is not more than 0.1 % and total content of the related substances is not more than 0.6 %.

$$\text{Amount (\%) of each related substance} = 100 \cdot F \cdot A_i / (A_N + \sum F \cdot A_i)$$

F: Relative response factor (0.12 for the related substance having the relative retention time of 0.73, 0.10 for the related substance having the relative retention time of 2.7, 0.25 for the related substance having the relative retention time of 0.93, 0.42 for the related substance having the relative retention time of 1.2, 0.94 for the related substance having the relative retention time of 0.85, 1.02 for the related substance having the relative retention time of 1.9, 0.91 for the related substance having the relative retention time of 2.6).

A_i: Peak area of each related substance.

A_N: Peak area of nabumetone.

Operating conditions

Detector, column, mobile phase and flow rate: proceed as directed in the operating conditions under Assay.

System suitability

System performance: Weigh a portion of Nabumetone RS and nabumetone related substance I RS, dissolve in acetonitrile, dilute suitably with acetonitrile to render the concentration of the solution of about 1 mg/mL and 1 μg/mL, respectively, and use this solution as the test solution for system suitability. When the procedure is run with 10 μL of the test solution for system suitability, the relative retention time for nabumetone peak and the related substance I peak is 1.0 and 0.9, respectively, with the resolution between the two peaks being not less than 1.5.

System repeatability: When the test is repeated 5 times with 10 μL each of the test solution for system suitability under the above operating conditions, the relative standard deviation of the areas of nabumetone peak is not more than 2.0 %.

Water Not more than 0.2 % (1 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.1 g of Nabumetone, add acetonitrile to make exactly 100 mL and use this solution as the test solution. Separately, weigh accurately about 0.1 g of Nabumetone RS, add acetonitrile to make exactly 100 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed in the Liquid Chromatography according to the following operating conditions and determine the area of nabumetone peak in the test solution, A_T, and in the standard solution, A_S.

$$\text{Amount (mg) of nabumetone } (C_{15}H_{16}O_2) = \text{Amount (mg) of Nabumetone RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatog-

raphy (4 μm in particle diameter).

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: A mixture of water and acetic acid (100) (999:1).

Mobile phase B: A mixture of acetonitrile and tetrahydrofuran (7:3)

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0	60	40
0-12	60	40
12-28	60→20	40→80
28-29	20→60	80→40
29-30	60	40

Flow rate: 1.3 mL/minute.

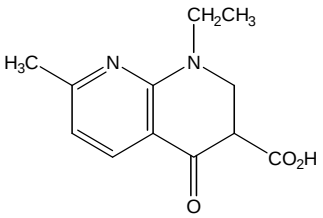
System suitability

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the areas of nabumetone peak is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Nalidixic Acid



$C_{12}H_{12}N_2O_3$: 232.24

1-Ethyl-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid [*389-08-2*]

Nalidixic Acid, when dried, contains not less than 98.5 % and not more than 101.0 % of nalidixic acid ($C_{12}H_{12}N_2O_3$).

Description Nalidixic Acid appears as white to pale yellow crystals or crystalline powder.

Nalidixic Acid is sparingly soluble in *N,N*-dimethylformamide, very slightly soluble in ethanol (99.5) and practically insoluble in water.

Nalidixic Acid dissolves in sodium hydroxide TS.

Identification (1) Determine the absorption spectra of solutions of Nalidixic Acid and Nalidixic Acid RS in 0.01 mol/L sodium hydroxide TS (1 in 200000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Nalidixic Acid and Nalidixic Acid RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 225 ~ 231 °C.

Purity (1) ***Chloride***—Take 2.0 g of Nalidixic Acid, add 50 mL of water, warm at 70 °C for 5 minutes, cool quickly and filter. To 25 mL of the filtrate, add 6 mL of dilute nitric acid and water to make 50 mL and perform the test with this solution as the test solution. Prepare the control solution with 0.35 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.012 %).

(2) ***Heavy metals***—Proceed with 1.0 g of Nalidixic Acid according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Dissolve 20 mg of Nalidixic Acid in 20 mL of 0.01 mol/L sodium hydroxide TS. Pipet 5 mL exactly of this solution, add water to make exactly 10 mL, and use this solution as the test solution. Pipet 2 mL of the test solution, add water to make exactly 1000 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the test solution and the standard solution according to the following conditions as directed under Liquid Chromatography, and determine each peak area by the automatic integration method. The area of the peak other than nalidixic acid with the test solution is not larger than the peak area of nalidixic acid with the standard solution, and the total area of the peaks other than the peak of nalidixic acid with the test solution is not larger than 2.5 times the peak area of nalidixic acid with the standard solution .

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

Column: A stainless steel column 4.6 mm in internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: Dissolve 6.24 g of sodium dihydrogen phosphate dihydrate in 950 mL of water, adjust the pH to 2.8 with phosphoric acid, and add water to make 1000 mL. To 300 mL of this solution add 200 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of nalidixic acid is about 19 minutes.

System suitability

Test for required detectability: Pipet 5 mL of the standard solution, and add water to make exactly 10

mL. Confirm that the peak area of nalidixic acid obtained with 10 μL of this solution is equivalent to 40 to 60 % of that with 10 μL of the standard solution.

System performance: Dissolve 25 mg of methyl parahydroxybenzoate in 100 mL of a mixture of water and methanol (1:1). To 1 mL of this solution add water to make 10 mL. To 5 mL of this solution add 5 mL of the standard solution. When the procedure is run with 10 μL of this solution under the above operating conditions, methyl parahydroxybenzoate and nalidixic acid are eluted in this order with the resolution between these peaks being not less than 13.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nalidixic acid is not more than 2.0 %.

Loss on Drying Not more than 0.2 % (1 g, 105 °C, 3 hours).

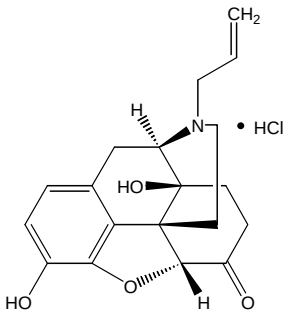
Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 0.3 g of Nalidixic Acid, previously dried, dissolve in 50 mL of *N,N'*-dimethylformamide, and titrate with 0.1 mol/L tetramethyl ammonium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Separately, to 50 mL of *N,N'*-dimethylformamide add 13 mL of a mixture of water and methanol (89 : 11), perform a blank determination with the solution, and make any necessary correction.

Each mL of 0.1 mL/L tetramethyl ammonium hydroxide VS = 23.22 mg of $C_{12}H_{12}N_2O_3$

Containers and Storage ***Containers***—Tight containers.

Naloxone Hydrochloride



$C_{19}H_{21}NO_4 \cdot HCl$: 363.84

(1*S*,5*R*,13*R*,17*S*)-10,17-Dihydroxy-4-(prop-2-en-1-yl)-12-oxa-4-azapentacyclo[9.6.1.01,13.05,17.07,18]octadeca-7(18),8,10-trien-14-one hydrochloride [*357-08-4*]

Naloxone Hydrochloride contains not less than 98.5 % and not more than 101.0 % of naloxone hydrochloride ($C_{19}H_{21}NO_4 \cdot HCl$), calculated on the dried basis.

Description Naloxone hydrochloride appears as white to yellow crystals or crystalline powder.
Naloxone Hydrochloride is freely soluble in water, soluble in methanol, slightly soluble in ethanol (99.5) and in acetic acid (100), and very slightly soluble in acetic anhydride.
Naloxone Hydrochloride is hygroscopic.
Naloxone Hydrochloride is gradually colored by light.

Identification (1) Determine the absorption spectra of solutions of Naloxone Hydrochloride and Naloxone Hydrochloride RS (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavelength.

(2) Determine the infrared spectra of Naloxone Hydrochloride and Naloxone Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Naloxone Hydrochloride (1 in 50) responds to the Qualitative Test (2) for chloride.

pH Dissolve 0.10 g of Naloxone Hydrochloride in 10 mL of freshly boiled and cooled water: the pH of the solution is between 4.5 and 5.5.

Specific Optical Rotation $[\alpha]_D^{25}$: -170 ~ -181° (0.25 g, water, 10 mL, 100 mm).

Purity ***Related substances***—Perform the test as rapidly as possible without exposure to light, using light-resistant container. Dissolve 80 mg of Naloxone Hydrochloride in 10 mL of methanol, and use this solution as the test solution. Pipet 1.0 mL of the test solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of ammonia saturated 1-butanol and methanol (20 : 1) to a distance of about 12 cm, and air-dry the plate. Spray evenly iron (III) chloride-potassium hexacyanoferrate (III) TS on the plate: the number of the spot other than the principal spot from the test solution is not more than one and it is not more intense than the spot from the standard solution.

Ammonia-saturated 1-butanol—Take 100 mL of 1-butanol, add 60 mL of ammonia solution (1 in 100), shake up and then use a layer of 1-butanol.

Loss on Drying Not more than 2.0 % (0.1 g, 105 °C,

5 hours, Use a desiccator (P_2O_5), for cooling).

Residue on Ignition Not more than 0.2 % (0.1 g).

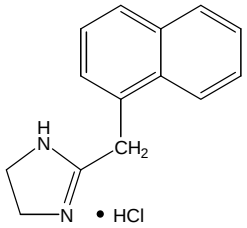
Assay Weigh accurately about 0.3 g of Naloxone Hydrochloride, and dissolve in 80 mL of acetic acid (100) by warming. After cooling, add 80 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 36.384 mg of $C_{19}H_{21}NO_4 \cdot HCl$

Containers and Storage ***Containers*—**Tight containers.

***Storage*—**Light-resistant.

Naphazoline Hydrochloride



$C_{14}H_{14}N_2 \cdot HCl$: 246.74

2-(Naphthalen-1-ylmethyl)-4,5-dihydro-1*H*-imidazole hydrochloride [*550-99-2*]

Naphazoline Hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of naphazoline hydrochloride ($C_{14}H_{14}N_2 \cdot HCl$).

Description Naphazoline Hydrochloride appears as white, crystalline powder, is odorless and has a bitter taste.
Naphazoline Hydrochloride is freely soluble in water, soluble in ethanol (95) or in acetic acid (100), very slightly soluble in acetic anhydride and practically insoluble in ether.

Melting point— 255 ~ 260 °C (with decomposition).

Identification (1) Take 10 mL of a solution of Naphazoline Hydrochloride (1 in 100), add 5 mL of bromine TS and boil: a deep purple color develops.

(2) Take 30 mL of a solution of Naphazoline Hydrochloride (1 in 100), add 2 mL of sodium hydroxide TS and extract with two 25 mL volumes of ether. Evaporate the combined ether extracts to dryness with the aid of a current of air. Dry the residue at 80 °C for 1 hour: the residue melts between 117 °C and 120 °C.

(3) Dissolve 20 mg of the residue obtained in (2) in 2 to 3 drops of dilute hydrochloric acid and 5 mL of water and add 2 mL of Reinecke salt TS: a red-purple, crystalline precipitate is formed.

(4) A solution of Naphazoline Hydrochloride (1 in 10) responds to the Qualitative Tests for chloride.

pH Dissolve 0.10 g of Naphazoline Hydrochloride in 10 mL of freshly boiled and cooled water: the pH of the solution is between 5.0 and 7.0.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Naphazoline Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Naphazoline Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

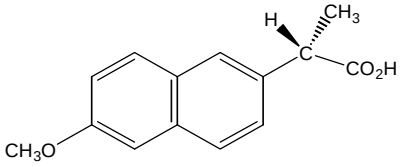
Assay Weigh accurately about 0.4 g of Naphazoline Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 24.674 mg of $C_{14}H_{14}N_2 \cdot HCl$

Containers and Storage ***Containers*—**Tight containers.

***Storage*—**Light-resistant.

Naproxen



$C_{14}H_{14}O_3$: 230.26

(*S*)-2-(6-Methoxynaphthalen-2-yl)propanoic acid [*22204-53-1*]

Naproxen, when dried, contains not less than 98.5 % and not more than 101.0 % of naproxen ($C_{14}H_{14}O_3$).

Description Naproxen appears as white crystals or crystalline powder and is odorless.
Naproxen is freely soluble in acetone, soluble in methanol, in ethanol (99.5) or in chloroform, sparingly sol-

uble in ether and practically insoluble in water.
Naproxen dissolves in sodium hydroxide TS.

Identification (1) Dissolve 10 mg of Naproxen in 5 mL of methanol, add 5 mL of water, then add 2 mL of potassium iodide TS and 5 mL of a solution of potassium iodate (1 in 100) and shake: a yellow to pale brown color is observed. To this solution, add 5 mL of chloroform and shake: a pale red-purple color is observed in the chloroform layer.

(2) Take 1 mL of a solution of Naproxen in ethanol (99.5) (1 in 300), add 4 mL of hydroxylamine perchlorate- ethanol (99.5) TS and 1 mL of *N*,*N*´-dicyclohexylcarbodiimide-ethanol (99.5) TS, shake well and allow to stand in lukewarm water for 20 minutes. After cooling, add 1 mL of iron (III) perchlorate hexahydrate- ethanol (99.5) TS and shake: a red-purple color is observed.

(3) Determine the absorption spectra of solutions of Naproxen and Naproxen RS in dehydrated methanol (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared spectra of Naproxen and Naproxen RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{25}$: +63.0 ~ +68.5° (0.1 g, after drying, chloroform, 10 mL, 100 mm).

Melting Point 154 ~ 158 °C.

Purity (1) ***Clarity and color of solution***—Prepare with 2.0 g of Naproxen in 20 mL of acetone: the solution is clear. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry: the absorbance at 400 nm is not more than 0.070.

(2) ***Heavy metals***—Proceed with 2.0 g of naproxen according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Prepare the test solution with 2.0 g of Naproxen according to Method 3 and perform the test (not more than 1 ppm).

(4) ***Related substances***—Perform the test without exposure to daylight, using light-resistant vessels. Dissolve 0.10 g of Naproxen in 10 mL of a mixture of ethanol (95) and chloroform (1 : 1) and use this solution as the test solution. Pipet 2.0 mL of the test solution and add a mixture of ethanol (95) and chloroform (1 : 1) to make exactly 100 mL. Pipet 5.0 mL of this solution, add a mixture of ethanol (95) and chloroform (1 : 1) to make exactly 50 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane, dichloromethane, tetrahydrofuran and acetic acid (100) (50 : 30 : 17 : 3) to a distance of about 12 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and the spot of the starting point from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

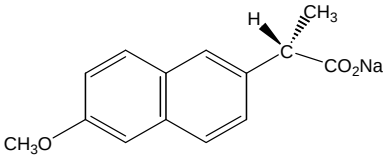
Assay Weigh accurately about 0.5 g of Naproxen, previously dried, add 100 mL of diluted methanol (4 in 5), dissolve by gentle warming, if necessary and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 23.026 mg of $C_{14}H_{14}O_3$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Naproxen Sodium



$C_{14}H_{13}NaO_3$: 252.24

Sodium (*S*)-2-(6-methoxynaphthalen-2-yl)propanoate
[*26159-34-2*]

Naproxen Sodium, when dried, contains not less than 98.0 % and not more than 102.0 % of naproxen sodium ($C_{14}H_{13}NaO_3$).

Description Naproxen Sodium is a white to pale yellowish, white crystalline powder.
Naproxen Sodium is soluble in water or in methanol, sparingly soluble in ethanol (95), very slightly soluble in acetone and practically insoluble in chloroform or in toluene.

Melting point—About 255 °C (with decomposition).

Identification (1) Determine the absorption spectra of solutions of Naproxen Sodium and Naproxen Sodium RS in methanol (1 in 40000) as directed under Ultraviolet-visible Spectrophotometry: both spectra ex-

hibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Naproxen Sodium and Naproxen Sodium RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibits similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{25}$: -15.3 ~ -17.0° (0.5 g, after drying, 0.1 mol/L sodium hydroxide TS, 10 mL, 100 mm).

Purity (1) ***Heavy metals***—Dissolve 1.0 g of Naproxen Sodium in 20 mL of water in a separatory funnel, add 5 mL of 1 mol/L hydrochloric acid TS and extract with successive 20 mL, 20 mL and 10 mL volumes of dichloromethane. Discard the dichloromethane extracts, proceed with the aqueous layer and perform the test according to Method 1. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Dissolve about 0.1 g of Naproxen Sodium, accurately weighed, in 5 mL of ethanol and use this solution as the test solution. Separately, dissolve a suitable quantity of Naproxen Sodium RS in methanol to obtain a standard solution having a known concentration of about 20 mg per mL. Pipet 1.0 mL, 3.0 mL and 5.0 mL of this solution and add methanol to make exactly 100 mL, respectively. Pipet 1.0 mL each of these solutions, add methanol to make exactly 10 mL and use these solutions as the standard solutions (1), (2) and (3). Perform the test with the test solution and the standard solutions (1), (2) and (3) as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solutions (1), (2) and (3) on a plate of silica gel for thin-layer chromatography. Then, develop the plate with a mixture containing toluene, tetrahydrofuran and acetic acid (100) (30 : 3 : 1) to a distance of about 15 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the R_f values of the principal spot obtained from the test solution and the standard solution are the same and the spots other than the principal spot from the test solution are not more intense than the spot obtained from the standard solution (3). The spots other than the principal spot are not more than 2.0 % of total spots, as compared to those obtained from the standard solutions (1), (2) and (3), respectively (0.1 %, 0.3 % and 0.5 %, respectively).

(3) ***Free naproxen***—Dissolve about 5.0 g of Naproxen Sodium in 25 mL of water in a separatory funnel and extract the solution with three 15 mL volumes of chloroform. Evaporate the combined extracts on a steam-bath to dryness. Dissolve the residue in 10 mL of a mixture of methanol and water (3 : 1), previously neutralized with 0.1 mol/L sodium hydroxide VS to the phenolphthalein end-point. Add phenolphthalein TS and titrate with 0.1 mol/L sodium hydroxide VS: not more than 2.2 mL is consumed (not more than 1.0 %).

Loss on Drying Not more than 1.0 % (1 g, in vacuum, 105 °C, 3 hours).

Assay Dissolve about 0.2 g of Naproxen Sodium, accurately weighed, in 50 mL of acetic acid (100) previously neutralized with 0.1 mol/L perchloric acid VS using 2 drops of *p*-naphtholbenzeine, if necessary and titrate with 0.1 mol/L perchloric acid VS.

Each mL of 0.1 mol/L perchloric acid VS
= 25.224 mg of $C_{14}H_{13}NaO_3$

Containers and Storage ***Containers***—Tight containers.

Naproxen Sodium Tablets

Naproxen Sodium Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of naproxen sodium ($C_{14}H_{13}NaO_3$: 252.25).

Method of Preparation Prepare as directed under Tablets, with Naproxen Sodium.

Identification (1) Transfer a portion of finely powdered Naproxen Sodium Tablets, equivalent to about 0.25 g of Naproxen Sodium, to a centrifuge tube and add 12 mL of water and 1 mL of hydrochloric acid: a white precipitate is produced. Centrifuge the mixture: the clear supernatant liquid responds to the Qualitative Tests for sodium salt.

(2) Prepare a mixture of the test solution and the standard solution (1 : 1) under the Assay and perform the test as directed under the Assay. The chromatogram so obtained exhibits two main peaks, corresponding to Naproxen and the internal standard.

Dissolution Test Perform the test with 1 tablet of Naproxen Sodium Tablets at 50 revolutions per minutes according to Method 2 under the Dissolution Test, using 900 mL of 0.1 mol/L of phosphate buffer (pH 7.4) as the dissolution solution. Take dissolved solution after 45 minutes from the start of the test, filter, if necessary and dilute with the dissolution solution to obtain a solution having a concentration of about 50 μg/mL of naproxen sodium ($C_{14}H_{13}NaO_3$) and use this solution as the test solution. Separately, weigh a portion of Naproxen Sodium RS, dissolve in the dissolution solution to make a solution containing 50 μg/mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 332 nm as directed under Ultraviolet-visible Spectrophotometry.
The dissolution rate of Naproxen Sodium Tablets in 45 minutes is not less than 80 %.

0.1 mol/L phosphate buffer (pH 7.4)—Prepared by

dissolving 2.62 g of monobasic sodium phosphate hydrate and 11.50 g of anhydrous dibasic sodium phosphate in water to make 1000 mL.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Naproxen Sodium Tablets and transfer an accurately weighed portion of the powder, equivalent to about 275 mg of naproxen sodium ($C_{14}H_{13}NaO_3$), to a volumetric flask. Add 10 mL of water and shake until it is completely dispersed. Dilute with acetonitrile to make exactly 100 mL and mix. Allow any insoluble matter to settle, then transfer 1.0 mL of the clear supernatant liquid to a volumetric flask, add 2.0 mL of the internal standard solution, dilute with the mobile phase to make exactly 100 mL, mix and use this solution as the test solution. Separately, dissolve an accurately weighed quantity of Naproxen Sodium RS in the mobile phase to obtain a solution having a known concentration of about 2.75 mg per mL. Transfer 1.0 mL of the resulting solution and 2.0 mL of the internal standard solution to a volumetric flask, dilute with the mobile phase to make 100 mL, mix and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and calculate the ratios, Q_T and Q_S, of the peak area of Naproxen Sodium to that of the internal standard, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of naproxen sodium } (C_{14}H_{13}NaO_3) = 10 \times C \times \frac{Q_T}{Q_S}$$

C: Concentration (μg/mL) of Naproxen Sodium RS in the standard solution.

Internal standard solution—Acetonitrile solution of butyrophenone (1 in 2000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of acetonitrile, water and glacial acetic acid (50 : 49 : 1).

Flow rate: 1.2 mL/minute.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, Naproxen Sodium and internal standard are eluted in this order with the resolution between their peaks being not less than 11.5.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of Naproxen Sodium to that of the internal standard is not more than 1.5 %.

Containers and Storage ***Containers***—Tight containers.

Naproxen Tablets

Naproxen Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of naproxen ($C_{14}H_{14}O_3$: 230.26).

Method of Preparation Prepare as directed under Tablets, with Naproxen.

Identification Prepare a mixture of the test solution and the standard solution (1 : 1) under the Assay and perform the test as directed under the Assay. The chromatogram so obtained exhibits two main peaks, corresponding to naproxen and the internal standard.

Dissolution Test Perform the test with 1 tablet of Naproxen Tablets at 50 revolutions per minutes according to Method 2 under the Dissolution Test, using 900 mL of 0.1 mol/L of phosphate buffer (pH 7.4) as the dissolution solution. Take dissolved solution after 45 minutes from the start of the test and filter. Dilute this solution with the phosphate buffer, if necessary and use this solution as the test solution. Separately, weigh accurately a portion of Naproxen RS, make the same concentration with the test solution and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 332 nm as directed under Ultraviolet-visible Spectrophotometry.
The dissolution rate of Naproxen Tablets in 45 minutes is not less than 80 %.

0.1 mol/L phosphate buffer (pH 7.4)—Prepared by dissolving 2.62 g of sodium phosphate, monobasic and 11.50 g of anhydrous dibasic sodium phosphate in water to make 1000 mL

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Naproxen Tablets. Weigh accurately a portion of the powder, equivalent to about 0.25 g of Naproxen ($C_{14}H_{14}O_3$) according to the labeled amount. Proceed as directed in the Assay under Naproxen Sodium Tablets. Seperately, weigh accurately a suitable amount of Naproxen RS. Instead of Naproxen Sodium RS, dissolve and add a mixture of acetonitrile and water (90 : 10) to obtain a solution having 2.5 mg of Naproxen in

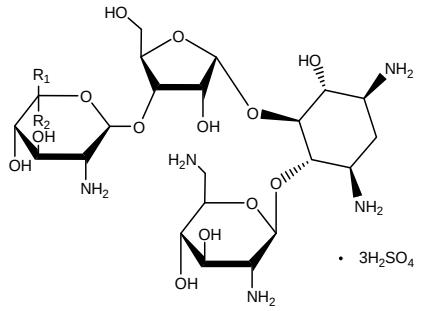
each mL.

$$\text{Amount (mg) of naproxen } (C_{14}H_{14}O_3) = 10 \times C \times \frac{Q_T}{Q_S}$$

C: Concentration (μg/mL) of Naproxen RS in the standard solution.

Containers and Storage ***Containers***—Tight containers.

Neomycin Sulfate



Neomycin B: R_1 = H R_2 = CH_2NH_2
Neomycin C: R_1 = CH_2NH_2 R_2 = H

$C_{23}H_{46}N_6O_{13}\cdot 3H_2SO_4$: 908.88

(2*R*,3*S*,4*R*,5*R*,6*R*)-5-Amino-2-(aminomethyl)-6-[(1*R*,2*R*,3*S*,4*R*,6*S*)-4,6-diamino-2-[(2*S*,3*R*,4*S*,5*R*)-4-[(2*R*,3*R*,4*R*,5*S*,6S)-3-amino-6-(aminomethyl)-4,5-dihydroxyoxan-2-yl]oxy-3-hydroxy-5-(hydroxymethyl)oxolan-2-yl]oxy-3-hydroxycyclohexyl]oxyoxane-3,4-diol; sulfuric acid [*1405-10-3*]

Neomycin Sulfate is the sulfate of a mixture of aminoglycoside substances having antibacterial activity produced by the growth of *Streptomyces fradiae* Waksman. Neomycin Sulfate, when dried, contains not less than 623 μg (potency) and not more than 740 μg (potency) of neomycin ($C_{23}H_{46}N_6O_{13}$: 614.65) per mg.

Description Neomycin Sulfate is a white to pale yellow powder.
Neomycin Sulfate is very soluble in water, and practically insoluble in ethanol (95).
Neomycin Sulfate is hygroscopic.

Identification (1) Dissolve 50 mg each of Neomycin Sulfate and Neomycin Sulfate RS in 1 mL of water and use these solutions as the test solution and the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 2 μL each of the test solution and the standard solution on a plate of silica gel for Thin-layer Chromatography. Develop the plate with a mixture of methanol, ammonia solution (28) and dichloromethane (3 : 2 : 1) to a distance of about 10 cm and air-dry the plate. Spray evenly ninhydrin acetone solution (1 in 50) and heat at 110 °C for 15 minutes: the principal spots obtained from the test solution and the standard solution are the same in R_f value.

(2) A solution of Neomycin Sulfate (1 in 20) responds to the Qualitative Tests (1) for sulfate.

Specific Optical Rotation $[\alpha]_D^{20}$: +53.5 ~ +59.0° (1 g calculated on the dried basis, water, 10 mL, 100 mm)

pH The pH of a solution obtained by dissolving 1.0 g of Neomycin Sulfate in 10 mL of water is between 5.0 and 7.5.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Neomycin Sulfate according to Method 2. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Arsenic***—Proceed with 1.0 g of Neomycin Sulfate according to Method 1 (not more than 2 ppm).

(3) ***Related substances***—Dissolve 0.63 g of Neomycin Sulfate in 5 mL of water and use this solution as the test solution. Pipet 1 mL of this solution, add water to make exactly 50 mL and use this solution as the standard solution. Perform the test with these solutions as directed uner the Thin-layer Chromatography. Spot 1 μL each of the test solution and the standard solution on a plate of silica gel for Thin-layer Chromatography. Develop the plate with a mixture of methanol, ammonia solution (28) and dichloromethane (3 : 2 : 1) to a distance of about 10 cm and air-dry the plate. Spray evenly ninhydrin acetone solution (1 in 50) and heat at 110 °C for 15 minutes: the spot having the R_f value of 0.4 obtained from the test solution is not more intense than the spot obtained from the standard solution.

Sterility Test It meets the requirement, when Neomycin Sulfate is used in a sterile preparation.

Loss on Drying Not more than 8.0 % (0.1 g, in vacuum, 60 °C, 3 hours).

Residue on Ignition Not more than 0.3 % (1 g)

Assay ***The Cylinder-plate method*** (1) Agar media for seed and base layer-

Peptone	6.0 g	Sodium chloride	2.5 g
Yeast extract	3.0 g	Glucose	1.0 g
Meat extract	1.5 g	Agar	15.0 g
Water	1000 mL		

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 7.8 to 8.0 after sterilization.

(2) Test organism and the suspension of test organism- *Staphylococcus aureus* ATCC 6538 P. The suspension of test organism has 80 % of transmission at 650 nm.

(3) Weigh accurately an amount of Neomycin Sulfate, previously dried, equivalent to about 50 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make the solution so that each mL contains 1 mg (potency), and use the solution as the test stock solution. Take exactly a suitable amount of the test stock solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 80.0 μg (potency) and 20.0 μg (potency), and use these solutions as the high concentration test solution and low concentration test solution, respectively. Separately, weigh accurately an amount of Neomycin Sulfate RS previously dried, equivalent to about 50 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make the solution so that each mL contains 1 mg (potency), and use the solution as the standard stock solution. Keep the standard stock solution at not exceeding 5 °C and use within 14 days. Take exactly a suitable amount of the standard stock solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 80.0 μg (potency) and 20.0 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively. Perform the test with these solutions according to the Cylinder-plate method (I 8) as directed under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Neomycin Sulfate Ointment

Neomycin Sulfate Ointment contains not less than 90.0 % and not more than 120.0 % of the labeled amount of neomycin ($C_{23}H_{46}N_6O_{13}$: 614.65).

Method of Preparation Prepare as directed under Ointments, with Neomycin Sulfate.

Identification Weigh a portion of Neomycin Sulfate Ointment, equivalent to about 35 mg of Neomycin Sulfate according to the labeled potency. Transfer to a separatory flask, add 50 mL of ether, shake vigorously and extract with 30 mL of 0.1 mol/L phosphate buffer (pH 3.0). To 5 mL of the extract, add 1 mL of ninhydrin TS and 0.5 mL of pyridine and boil for 10 minutes: a blue-purple color develops.

Water Not more than 1.0 % (1 g, volumetric titration, direct titration)

Assay Perform the test as directed in the Assay under Neomycin Suflate. Prepare the test solution as follows. Weigh accurately a portion of Neomycin Sulfate Ointment, equivalent to about 0.1 g (potency), and transfer to a separatory funnel. Add 50 mL of ether, shake thoroughly and extract with three 25 mL volumes of 0.1 mol/L phosphate buffer (pH 8.0). Combine the extracts and make a solution of suitable concentration with 0.1 mol/L phosphate buffer (pH 8.0). Pipet a suitable amount of this solution, dilute with 0.1 mol/L phosphate buffer (pH 8.0) to make the concentration of (3) and use this solution as the test solution.

Containers and Storage ***Containers***—Tight containers.

Neomycin Sulfate·Polymyxin B Sulfate Ophthalmic Solution

Neomycin Sulfate·Polymyxin B Sulfate Ophthalmic Solution contains not less than 90.0 % and not more than 120.0 % of the labeled amount of neomycin ($C_{23}H_{46}N_6O_{13}$: 614.65) and Polymyxin B.

Method of Preparation Prepare as directed under Ophthalmic Solutions, with Neomycin Sulfate and Polymyxin B.

Identification Weigh a suitable amount of Neomycin Sulfate·Polymyxin B Sulfate Ophthalmic Solution, Neomycin Sulfate RS and Polymyxin B Sulfate RS and dissolve in water to make solutions containing 1 mg (potency) of Neomycin Sulfate or 200 units (potency) of Polymyxin B Sulfate per mL, and use these solutions as the test solution and the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot the test solution and the standard solution on a plate of silica gel for Thin-layer Chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (60 : 20 : 20) and air-dry the plate. Spray evenly a solution prepared by adding a small amount of pyridine to 1 % ninhydrin solution in 1-butanol: the spots obtained from the test solution and the standard solution are the same in R_f value.

pH 5.2 ~ 5.8.

Sterility Test It meets the requirement.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Ophthalmic Solutions It meets the requirement.

Assay (1) ***The Standard curve method for Neomycin Sulfate*** (i) Agar media for seed and base layer- Use the medium in I 2 1) (2) ① under Microbial Assay for Antibiotics.

(ii) Agar medium for transferring test organisms- Use the medium in I 2 1) (2) ① under Microbial Assay for Antibiotics. Adjust the pH to 6.5 to 6.6.

(iii) Test organism- *Staphylococcus epidermidis* ATCC 12228

(iv) Pipet a suitable amount of Neomycin Sulfate·Polymyxin B Sulfate Ophthalmic Solution according to the labeled potency, transfer to a separatory funnel, dilute with 0.1 mol/L phosphate buffer (pH 8.0) to make a solution containing 1.00 μg (potency) per mL and use this solution as the test solution. Separately, weigh accurately a suitable amount of Neomycin Sulfate RS and dissolve in 0.1 mol/L phosphate buffer (pH 8.0) to make a standard stock solution containing 1.0 mg (potency) per mL. Store this standard stock solution at not exceeding 5 °C and use within 14 days. Pipet a suitable amount of this standard stock solution and dilute with 0.1 mol/L phosphate buffer (pH 8.0) to make solutions containing 0.64, 0.80, 1.00, 1.25 and 1.56 μg (potency) per mL respectively, and use these solutions as the standard solution. Use the solution containing 1.00 μg (potency) per mL as the standard mid-diluted solution. Perform the test with the test solution, the standard solutions and the standard mid-diluted solution as directed in II 4 under Microbial Assay for Antibiotics.

(2) ***The Standard curve method for Polymyxin B Sulfate*** (i) Agar medium for transferring test organisms- Use the medium in I 2 1) (3) ① under Microbial Assay for Antibiotics. Adjust the pH to 6.5 to 6.6.

(ii) Agar medium for base layer-

Casein pancreatic digest	17.0 g
Potassium dihydrogen phosphate	2.5 g
Papain digest of soybean meal	3.0 g
Glucose	2.5 g
Sodium chloride	5.0 g
Agar	20.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 7.3 after sterilization.

(iii) Agar medium for seed layer- To the agar medium for base layer from (ii), add 12.0 g of agar and 10.0 g of polysorbate 80 (previously boiled). Adjust the pH of the solution so that it will be 7.3 after sterilization.

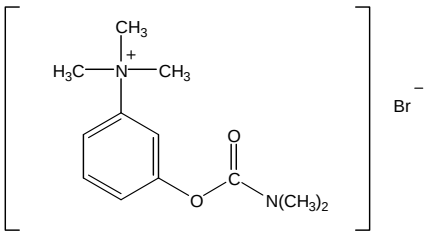
(iv) Test organism- *Bordetella bronchiseptica* ATCC 4617

(v) Test organism suspension- Preserve the test organism on the medium of (i) and transfer to a new slant once every week. Transfer the test organism to a new slant and incubate at 37 °C for 24 hours. Collect the resulting growth by washing with 3 mL of sterile isotonic sodium chloride injection. Transfer this suspension to a Roux bottle containing 300 mL of the medium of (i), spread evenly using a sterile glass spreader and incubate at 37 °C for 24 hours. Collect the growth on the surface of the agar by washing with 500 mL of sterile isotonic sodium chloride injection. Render the transmission of this suspension 25 % at 580 nm, using a suitable spectrophotometer. Add 0.1 mL of this adjusted suspension to 100 mL of the agar medium of (iii) and use this as the test organism suspension.

(vi) Pipet a suitable amount of Neomycin Sulfate·Polymyxin B Sulfate Ophthalmic Solution according to the labeled potency of Polymyxin B. Dilute with 10 % phosphate buffer (pH 6.0) to make a solution containing 10.0 units (potency) per mL and use this solution as the test solution. Separately, weigh accurately about 50 mg of Polymyxin B Sulfate RS, dissolve in 20 mL of sterile distilled water and add 10 % phosphate buffer (pH 6.0) to make a standard stock solution containing 10000 units (potency) per mL. Keep this standard stock solution at not exceeding 5 °C and use within 14 days. Pipet a suitable amount of this standard stock solution and dilute with 10 % phosphate buffer (pH 6.0) to make solutions containing 6.4, 8.0, 10.0, 12.5 and 15.6 units (potency) per mL respectively, and use these solutions as the standard solutions. Use the solution containing 10.0 units (potency) per mL as the standard mid-diluted solution. To each standard solution, add a portion of neomycin equivalent to the potency of neomycin in a solution containing 10.0 units (potency) of Polymyxin B per mL. Perform the test with the test solution, the standard solutions and the standard mid-diluted solution as directed in II 4 under Microbial Assay for Antibiotics.

Containers and Storage ***Containers*—**Tight containers.

Neostigmine Bromide



$C_{12}H_{19}BrN_2O_2$: 303.20

3-{[(Dimethylamino)carbonyl]oxy}-*N,N,N*-trimethylbenzenaminium bromide [*114-80-7*]

Neostigmine Bromide contains not less than 98.0 % and not more than 102.0 % of neostigmine bromide ($C_{12}H_{19}BrN_2O_2$), calculated on the anhydrous basis.

Description Neostigmine Bromide is a white, crystalline powder, is odorless and has a bitter taste.
Neostigmine Bromide is very soluble in water, soluble in ethanol (95) and practically in soluble in ether.
A solution of Neostigmine Bromide is neutral.

Identification (1) Determine the infrared spectra of Neostigmine Bromide and Neostigmine Bromide RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) A solution of Neostigmine Bromide (1 in 50) responds to the Qualitative Tests for bromide.

Melting Point 171 ~ 176 °C (with decomposition).

Purity ***Sulfate***—Dissolve 0.25 g of Neostigmine Bromide in 10 mL of water, add 1 mL of 3 mol/L hydrochloric acid TS and 1 mL of barium chloride TS: no turbidity is produced immediately.

Loss on Drying Not more than 2.0 % (1 g, 105 °C, 3 hours).

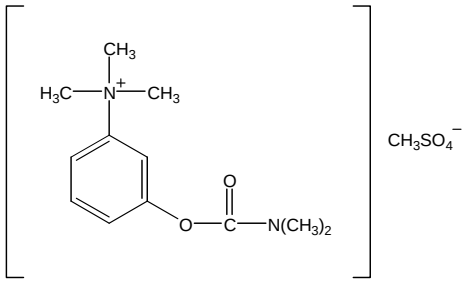
Residue on Ignition Not more than 0.15 % (1 g).

Assay Weigh accurately about 0.75 g of Neostigmine Bromide, dissolve in 70 mL of glacial acetic acid and 20 mL of mercury (II) acetate TS and titrate with 0.1 mol/L hyperchloric acid VS to blue (indicator: methylrosaniline chloride TS 4 drops). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L hyperchloric acid VS
= 30.320 mg of $C_{12}H_{19}BrN_2O_2$

Containers and Storage ***Containers***—Tight containers

Neostigmine Methylsulfate



$C_{13}H_{22}N_2O_6S$: 334.39

3-{[(Dimethylamino)carbonyl]oxy}-*N,N,N*-trimethylbenzenaminium; methyl sulfate [*51-60-5*]

Neostigmine Methylsulfate, when dried, contains not less than 98.0 % and not more than 102.0 % of neostigmine methylsulfate ($C_{13}H_{22}N_2O_6S$).

Description Neostigmine Methylsulfate is a white, crystalline powder.
Neostigmine Methylsulfate is very soluble in water and freely soluble in ethanol (95) and in acetonitrile.

Identification (1) Determine the absorption spectra of solutions of Neostigmine Methylsulfate and Neostigmine Methylsulfate RS (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Neostigmine Methylsulfate and Neostigmine Methylsulfate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 145 ~ 149 °C.

pH Dissolve 1.0 g of Neostigmine Methylsulfate in 10 mL of freshly boiled and cooled water. The pH of this solution is between 3.0 and 5.0.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Neostigmine Methylsulfate in 10 mL of water: the solution is clear and colorless.

(2) ***Sulfate***—Dissolve 0.20 g of Neostigmine Methylsulfate in 10 mL of water, add 1 mL of dilute hydrochloric acid and 1 mL of barium chloride TS: no turbidity is observed immediately.

(3) ***Dimethylaminophenol***—Dissolve 0.10 g of Neostigmine Methylsulfate in 5 mL of water, add 1 mL of sodium hydroxide TS and while cooling with ice, add 1 mL of diazobenzenesulfonic acid TS: no color is observed.

Loss on Drying Not more than 1.0 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 25 mg each of Neostigmine Methysullfate and Neostigmine Methylsulfate RS, previously dried, dissolve each in the mobile phase to make exactly 50 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under the Liqiud Chromatography according to the following operating conditions and determine the peak areas, A_T and A_S, neostigmine in the test and the standard solutions, respectively.

Amount (mg) of neostigmine methylsulfate ($C_{13}H_{22}N_2O_6S$: 334.39) = Amount (mg) of Neostigmine Methylsulfate RS $\times \dfrac{A_T}{A_S}$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 259 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: Dissolve 3.12 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, adjust the pH to 3.0 with phosphoric acid, and add 0.871 g of sodium 1-pentanesulfonate to dissolve. To

890 mL of this solution, add 110 mL of acetonitile.

Flow rate: Adjust the flow rate so that the retention time of Neostigmine is about 9 minutes.

System suitability

System performance: Dissolve 25 mg of Neostigmine Methylsulfate and 4 mg of dimethylaminophenol in 50 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, dimethylaminophenol and neostigmine are eluted in this order with a resolution between their peaks being not less than 6.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of neostigmine is not more than 1.0 %.

Containers and Storage ***Containers*—**Tight containers

Neostigmine Methylsulfate Injection

Neostigmine Methylsulfate Injection is an aqueous solution for injection. Neostigmine Methylsulfate Injection contains not less than 93.0 % and not more than 107.0 % of the labeled amount of neostigmine methylsulfate ($C_{13}H_{22}N_2O_6S$: 334.39).

Method of Preparation Prepare as directed under Injections, with Neostigmine Methylsulfate.

Description Neostigmine Methylsulfate Injection is a clear, colorless liquid. Neostigmine Methylsulfate Injection is gradually affected by light.

pH—5.0 ~ 6.5

Identification Transfer a volume of Neostigmine Methylsulfate Injection, equivalent to 5 mg of Neostigmine Methylsulfate according to the labeled amount, if necessary, add water to make 10 mL. Determine the absorption specra of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maxima between 257 nm and 261 nm.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 5 EU/mg of Neostigmine Methylsulfate.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

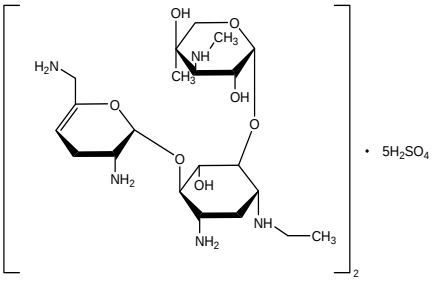
Assay Use Neostigmine Methylsulfate Injection as the test solution. Separately, weigh accurately about 25 mg of Neostigmine Methylsulfate RS, previously dried at 105 °C for 3 hours and dissolve in the mobile phase to make exactly 50 mL. And use this solution as the standard solution. Proceed as directed in the Assay under Neostigmine Methylsulfate.

$$\text{Amount (mg) of neostigmine methylsulfate } (C_{13}H_{22}N_2O_6S) = \text{Amount (mg) of Neostigmne Methylsulfate RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers*—**Hermetic containers.

***Storage*—**Light-resistant.

Netilmicin Sulfate



$(C_{21}H_{41}N_5O_7)_2 \cdot 5H_2SO_4$: 1441.55

(2*R*,3*R*,4*R*,5*R*)-2-[(1*S*,2*S*,3*R*,4*S*,6*R*)-4-Amino-3-[[(2*S*,3*R*)-3-amino-6-(aminomethyl)-3,4-dihydro-2*H*-pyran-2-yl]oxy]-6-(ethylamino)-2-hydroxycyclohexyl]oxy-5-methyl-4-(methylamino)oxane-3,5-diol; sulfuric acid [*56391-57-2*]

Netilmicin Sulfate is the sulfate of a derivative of sisomicin.

Netilmicin Sulfate contains not less than 595 μg (potency) and not more than 720 μg (potency) of netilmicin ($C_{21}H_{41}N_5O_7$: 475.58) per mg, calculated on the dried basis

Description Netilmicin Sulfate appears as white to pale yellow powder.

Netilmicin Sulfate is very soluble in water and practically insoluble in ethanol (95) and in ether.

Netilmicin Sulfate is hygroscopic.

Identification (1) Dissolve 30 mg of Netilmicin Sulfate in 3 mL of water and add 0.2 mL of bromine TS: the color of the solution disappears immediately.

(2) Weigh 5 mg each of Netilmicin Sulfate and Netilmicin Sulfate RS, dissolve in 5 mL of water and

use these solutions as the test solution and the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for Thin-layer Chromatography. Develop the plate with a mixture of methanol, chloroform, ammonia solution (28) and acetone (2:2:1:1) to a distance of about 15 cm and air-dry the plate. Spray evenly 0.2 % ninhydrin-water saturated 1-butanol TS and heat at about 100 °C for about 5 minutes: the principal spots from the test solution and the standard solution exhibit a red-purple to red-brown color and the same R_f value.

(3) A solution of Netilmicin Sulfate (1 in 100) responds to the Qualitative Tests (1) for sulfate.

Specific Optical Rotation $[\alpha]_D^{20}$: +88 ~ +96° (30 mg calculated on the dried basis, water, 10 mL, 100 mm)

pH The pH of a solution obtained by dissolving 0.2 g of Netilmicin Sulfate in 10 mL of water is between 3.5 and 5.5.

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Netilmicin Sulfate in 5 mL of water: the solution is clear and colorless to light yellow.

(2) ***Heavy metals***—Proceed with 1.0 g of Netilmicin Sulfate according to Method 2. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Weigh a portion of Netilmicin Sulfate equivalent to 50 mg calculated on the dried basis, dissolve in water to make 5 mL and use this solution as the test solution, Pipet 0.5 mL, 1 mL, 1.5 mL and 3 mL of the test solution, add water to each to make exactly 50 mL and use these solutions as the standard solutions (1), (2), (3) and (4), respectively. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL of each of the standard solutions (1), (2), (3) and (4) on a plate of silica gel for Thin-layer Chromatography. Develop the plate with a mixture of methanol, chloroform, ammonia solution (28) and acetone (2:2:1:1) to a distance of about 10 cm and air-dry the plate. Spray 0.2 % ninhydrin-water saturated 1-butanol TS and heat at about 100 °C for 5 minutes: the spots other than the principal spot obtained from the test solution are not more intense than the spot obtained from the standard solution (3). The total intensity of spots other than the principal spot obtained from the test solution is not more intense than the spot obtained from the standard solution (4) (not more than 6 %).

Sterility Test It meets the requirement, when Netilmicin Sulfate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.50 EU/mg of netilmicin, when Netilmicin Sulfate is used in a sterile preparation.

Loss on Drying Not more than 15.0 % (0.15 g, not more than 0.67 kPa, 110 °C, 3 hours). Avoid moisture absorption when taking the sample.

Residue on Ignition Not more than 1.0 % (1 g).

Assay Weigh accurately about 50 mg each of Netilmicin Sulfate and Netilmicin Sulfate RS, dissolve in the mobile phase to make exactly 50 mL each, and use these solutions as the test solution and the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the peak area of netilmicin in the test solution and the standard solution, A_T and A_S.

$$\text{Amount [μg (potency)] of netilmicin } (C_{16}H_{17}N_3O_5S) = \text{Amount [μg (potency)] of Netilmicin Sulfate RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 205 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of diluted phosphoric acid (5 in 1000) added to 22.02 g of sodium 1-heptane sulfonate to make 1000 mL, with acetonitrile (620:380)

Flow rate: 1 mL/minute

System suitability

System performance: Weigh accurately a suitable amount of Netilmicin Sulfate and Sisomicin Sulfate RS to make a solution containing 1 mg of each per mL, and use this solution as the system suitability solution. When the procedure is run with 20 μL of the system suitability solution under the above operating conditions, the resolution between the peaks of sisomicin and netilmicin is not less than 1. When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates is not less than 3000 with the symmetry factor being not more than 2.

System repeatability: When the test is repeated 5 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of netilmicin is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, under nitrogen or argon atmosphere, and not exceeding 5 °C.

Netilmicin Sulfate Injection

Netilmicin Sulfate Injection contains not less than 90.0 % and not more than 120.0 % of the labeled amount of netilmicin ($C_{21}H_{41}N_5O_7$: 475.58).

Method of Preparation Prepare as directed under Injections, with Netilmicin Sulfate.

Description Netilmicin Sulfate Injection appears as colorless to pale yellow, clear liquid.

Identification Perform the test as directed in the Identification (2) under Netilmicin Sulfate.

pH 3.5 ~ 6.0.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.50 EU/mg (potency) of netilmicin.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

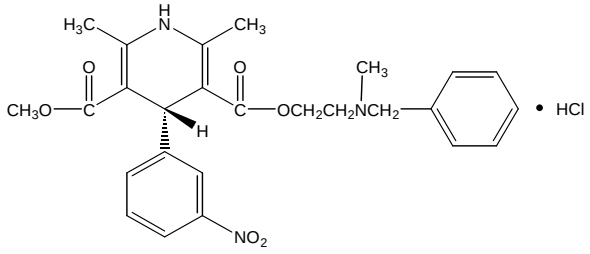
Determination of Volume of Injection in Containers It meets the requirement.

Assay Perform the test as directed in the Assay under Netilmicin Sulfate. Pipet an amount of Netilmicin Sulfate Injection equivalent to about 0.100 g (potency) according to the labeled potency, dissolve in the mobile phase to make exactly 100 mL and use this solution as the test solution.

$$\text{Amount [μg (potency)] of netilmicin } (C_{16}H_{17}N_3O_5S) = \text{Amount [μg (potency)] of Netilmicin Sulfate RS} \times \frac{A_T}{A_S} \times 2$$

Containers and Storage ***Containers***—Hermetic containers.

Nicardipine Hydrochloride



and enantiomer

$C_{26}H_{29}N_3O_6 \cdot HCl$: 515.99

3-(2-(Benzyl(methyl)amino)ethyl) 5-methyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate hydrochloride [*54527-84-3*]

Nicardipine hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of nicardipine hydrochloride ($C_{26}H_{29}N_3O_6 \cdot HCl$).

Description Nicardipine Hydrochloride appears as pale greenish yellow, crystalline powder.
Nicardipine Hydrochloride is freely soluble in methanol or in acetic acid (100), sparingly soluble in ethanol (95) and insoluble in water, in acetonitrile or in acetic anhydride.
A solution of Nicardipine Hydrochloride in methanol (1 in 20) shows no optical rotation.
Nicardipine Hydrochloride is gradually affected by light.

Identification (1) Determine the absorption spectra of solutions of Nicardipine Hydrochloride and Nicardipine Hydrochloride RS in ethanol (99.5) (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Nicardipine Hydrochloride and Nicardipine Hydrochlorid RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both exhibit similar intensity of absorption at the same wavenumbers.

(3) Dissolve 20 mg of Nicardipine Hydrochloride in 10 mL of water and 3 mL of nitric acid: the solution responds to the Qualitative Tests for chloride.

Melting Point 167 ~ 171 °C.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Nicardipine Hydrochloride according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Perform the test without exposure to daylight, using light-resistant vessels. Dissolve 0.10 g of Nicardipine Hydrochloride in 50 mL of the mobile phase and use this solution as the test solution. Pipet 1.0 mL of the test solution, add the mobile phase to make exactly 50 mL, then take 1.0 mL of this solution, add the mobile phase to make exactly 10 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peak of nicardipine from the test solution is not larger than the peak area of nicardipine from the standard solution and the total area of each peak other

than the peak of nicardipine from the test solution is not more than twice the peak area of nicardipine from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30 °C.

Mobile phase: A mixture of a solution of perchloric acid (43 in 50000) and acetonitrile (3 : 2).

Flow rate: Adjust the flow rate so that the retention time of nicardipine is about 6 minutes.

System suitability

Detection sensitivity: Take 2.0 mL of the standard solution add the mobile phase to make exactly 20 mL. Adjust the peak area of nicardipine obtained from 20 μL of this solution is equivalent to 8 % to 12 % of the peak area of nicardipine.

System performance: Dissolve 2 mg each of Nicardipine Hydrochloride and Nifedipine RS in 50 mL of the mobile phase. When the procedure is run with 10 μL of this solution according to the above operating conditions, nicardipine and nifedipine are eluted in this order with the resolution between their peaks being not less than 3.0.

System repeatability: When test is repeated 6 times with 10 μL each of the standard solution according to the above operating conditions, the relative standard deviation of the peak area of nicardipine is not more than 3.0 %.

Time span of measurement: About four times as long as the retention time of Nicardipine after the solvent peak.

Loss on Drying Not more than 1.0 % (1 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

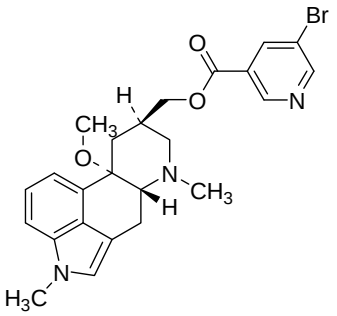
Assay Perform the test without exposure to day-light, using light-resistant vessels. Weigh accurately about 0.9 g of Nicardipine Hydrochloride, previously dried, dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 51.60 mg of $C_{26}H_{29}N_3O_6 \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Nicergoline



$C_{24}H_{26}BrN_3O_3$: 484.39

[(6a*R*,9*R*,10a*S*)-10a-Methoxy-4,7-dimethyl-6a,8,9,10-tetrahydro-6H-indolo[4,3-fg]quinoline-9-yl]methyl-5-bromopyridine-3-carboxylate [*27848-84-6*]

Nicergoline, when dried, contains not less than 98.5 % and not more than 101.0 % of nicergoline ($C_{24}H_{26}BrN_3O_3$).

Description Nicergoline occurs as white to pale yellow crystals or crystalline powder.

Nicergoline is sparingly soluble in ethanol (99.5) and in acetonitrile and in acetic anhydride, and practically insoluble in water.

Nicergoline gradually colored to palet brown by light.

Melting point—About 136 °C (with decomposition)

Identification (1) Determine the absorption spectra of the solution of Nicergoline and Nicergoline RS in ethanol (99.5) (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry and compare these spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Nicergoline and Nicergoline RS as directed in the potassium bromide disk method under Infrared Spectrophotometry and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +5.2 ~ + 6.2° (after drying, 0.5 g, ethanol (95), 10 mL, 100 mm).

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Nicergoline according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Dissolve 25 mg of Nicergoline in 25 mL of acetonitrile, and use this solution as the test solution. Pipet 1.0 mL of test solution, and add acetonitrile to make exactly 100 mL. Pipet 10 mL of this solution, add acetnotrile to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatog-

raphy according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the related retention time of about 0.5 with respect to nicergoline, is not larger than 4 times the peak area of nicergoline from the standard solution, and the area of the peak other than nicergoline and other than the peak mentioned above is not larger than 2.5 times the peak area of nicergoline from the standard solution. The peak which area is larger than the peak area of nicergoline from the standard solution is not more than two peaks, and the total peak area of the peaks other than the peak area of nicergoline from the test solution is not larger than 7.5 times the peak area of nicergoline from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 288 nm).

Column: A stainless steel column 4.6 mm in internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 7.0. To 350 mL of this solution add 350 mL of methanol and 300 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of nicergoline is about 25 minutes.

System suitability

Test for required detectability: To 1 mL of the test solution add acetonitrile to make exactly 50 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add acetonitrile to make exactly 100 mL. Confirm that the peak area of nicergoline obtained with 20 μL of this solution is equivalent to 3 to 7 % of that obtained with 20 μL of the solution for system suitability test.

System performance: When the procedure is run with 20 μL of the test solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nicergoline are not less than 8000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the test solution under the above operating conditions, the relative standard deviation of the peak area of nicergoline is not more than 4.0 %.

Time span of measurement: About 2 times as long as the retention time of nicergoline beginning after the solvent peak

Loss on Drying Not more than 0.5 % (2 g, in vacuum, 60 °C, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.4 g of Nicergoline, previously dried, add 10 mL of acetic anhydride, and warm to dissolve. After cooling, add 40 mL of nitrobenzene and titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes to blue-green from red through a blue-purple (indicator: 10 drops of neutral red TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid
= 24.22 mg $C_{24}H_{26}BrN_3O_3$

Containers and Storage ***Containers*—**Well-closed containers.

***Storage*—**Light-resistant.

Nicergoline Tablets

Nicergoline Tablets contain not less than 95.0 % and not more than 105.0 % of the labeled amount of nicergoline ($C_{24}H_{26}BrN_3O_3$: 484.39).

Method of Preparation Prepare as directed under Tablets, with Nicergoline.

Identification Take a quantity of powdered Nicergoline Tablets, equivalent to 10 mg of nicergoline according to the labeled amount, add 20 mL of ethanol (99.5), shake vigorously for 10 minutes, and filter through a membrane filter with a pore size of not exceeding 0.45 μm. To 2 mL of the filtrate add ethanol (99.5) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 226 nm and 230 nm, and between 286 nm and 290 nm.

Purity (1) ***Related substances***—Perform the test with 20 μL of the test solution obtained in the Assay as directed under Liquid Chromatography according to the following operating conditions. Determine each peak area by the automatic integration method, and calculate the amount of substances other than nicergoline by the area percentage method: the total amount of them is not more than 2.0 %.

Operating conditions

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: To 1 mL of the standard solution obtained in the Assay add a mixture of acetonitrile and water (17 : 3) to make 50 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, add the mixture of acetonitrile and water (17 : 3) to make exactly 100 mL. Confirm that the peak area of

nicergoline obtained with 20 μL of this solution is 3 to 7 % of that with 20 μL of the solution for system suitability test.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of nicergoline is not more than 1.5 %.

Time span of measurement: About 2 times as long as the retention time of nicergoline beginning after the solvent peak.

Disintegration Test It meets the requirement.

Uniformity of Dosage Units It meets the requirement when the content uniformity test is performed according to the following procedure.

To 1 tablet of Nicergoline Tablets add exactly 25 mL of diluted ethanol (4 in 5), disperse to fine particles with the aid of ultrasonic wave and shake for 5 minutes. Centrifuge this solution for 10 minutes, pipet 4 mL of the supernatant liquid, add diluted ethanol (4 in 5) to make exactly 25 mL, and use this solution as the test solution. Separately, weigh accurately about 10 mg of Nicergoline RS, previously dried in vacuum at 60 °C for 2 hours, and dissolve in exactly 25 mL of diluted ethanol (4 in 5). Pipet 4 mL of this solution, add diluted ethanol (4 in 5) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances at 288 nm, A_{T1} and A_{S1}, and at 340 nm, A_{T2} and A_{S2}, of the sample solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of nicergoline } (C_{24}H_{26}BrN_4O_3) = \text{Amount (mg) of Nicergoline RS} \times \frac{A_{T1} - A_{T2}}{A_{S1} - A_{S2}} \times \frac{1}{2}$$

Assay Weigh accurately the mass of not less than 20 Nicergoline Tablet, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of nicergoline ($C_{24}H_{26}BrN_3O_3$), add exactly 20 mL of a mixture of acetonitrile and water (17 : 3), vigorously shake for 10 minutes, centrifuge for 10 minutes, and use the supernatant liquid as the test solution. Separately, weigh accurately about 20 mg of Nicergoline RS, previously dried in vacuum at 60 °C for 2 hours, dissolve in exactly 20 mL of the mixture of acetonitrile and water (17 : 3), and use this solution as the standard solution. Perform the test with exactly 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of nicergoline.

$$\text{Amount (mg) of nicergoline } (C_{24}H_{26}BrN_4O_3) = \text{Amount (mg) of Nicergoline RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 288 nm).

Column: A stainless steel column, 4.6 mm in internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 7.0 with triethylamine. To 350 mL of this solution add 350 mL of methanol and 300 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of nicergoline is about 25 minutes.

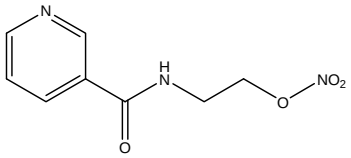
System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nicergoline are not less than 8000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nicergoline is not more than 1.0 %.

Containers and Storage ***Containers*—**Tight containers.

Nicorandil



$C_8H_9N_3O_4$: 211.17

2-[(Pyridin-3-ylcarbonyl)amino]ethyl nitrate
[*65141-46-0*]

Nicorandil contains not less than 98.5 % and not more than 101.0 % of nicorandil ($C_8H_9N_3O_4$), calculated on the anhydrous basis.

Description Nicorandil appears as white crystals.

Nicorandil is freely soluble in methanol, in ethanol (99.5) and in acetic acid (100), sparingly soluble in acetic anhydride and practically insoluble in water.

Melting point—About 92 °C (with decomposition)

Identification (1) Determine the absorption spectra of the solution of Nicorandil and Nicorandil RS in water (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry and compare these spectra: both

spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Nicorandil and Nicorandil RS as directed in the potassium bromide disk method under Infrared Spectrophotometry and compare these spectra: both spectra exhibit the same intensities of absorption at the same wave numbers.

Purity (1) ***Sulfate***—Dissolve 2.0 g of Nicorandil in 20 mL of dilute ethanol, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS, 20 mL of dilute ethanol and 1 mL of dilute hydrochloric acid VS, 20 mL of dilute ethanol and 1 mL of dilute hydrochloric acid, and dilute with water to make 50 mL (not more than 0.010 %).

(2) ***Heavy metals***—Proceed with 0.2 g of Nicorandil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Dissolve 20 mg of Nicorandil in 10 mL of the mobile phase and use this solution as the test solution. Perform the test with 10 μL of the test solution as directed under Liquid Chromatography according to the following operating conditions, and determine each peak area by the automatic integration method: the peak area of *N*-(2-hydroxyethyl)isonicotinamide nitric ester, having the relative retention time of about 0.86 with respect to Nicorandil, is not more than 0.5 % of the peak area of Nicorandil, the area of all other peaks is less than 0.1 %, and the sum area of the peaks other than nicorandil and *N*-(2-hydroxyethyl)isonicotinamide nitric ester is not more than 0.25 % of the total peak area.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.0 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: A mixture of water, tetrahydrofuran, triethylamine and trifluoroacetic acid (982:10:5:3)

Flow rate: Adjust the flow rate so that the retention time of nicorandil is about 18 minutes.

System suitability

Test for required detectability: Pipet 1 mL of the test solution, add the mobile phase to make exactly 500 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of nicorandil obtained with 10 μL of this solution is equivalent to 2 to 8 % of that with 10 μL of the solution for system suitability test.

System performance: Dissolve 10 mg of *N*-(2-hydroxyethyl)isonicotinamide nitric ester in the mobile phase to make 100 mL. To 1 mL of this solution add 10 mL of the test solution. When the procedure is run with this solution under the above operating conditions, *N*-(2-hydroxyethyl)isonicotinamide nitric ester and nicorandil are eluted in this order with the resolution between these peaks being not less than 3.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of nicorandil is not more than 1.5 %.

Time span of measurement: About 3 times as long as the retention time of nicorandil beginning after the solvent peak.

Water Not more than 0.1 % (2 g, volumetric titration, direct titration).

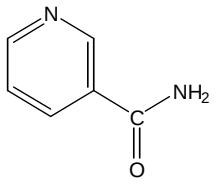
Residue on Ignition Not more than 0.1 % (1 g)

Assay Weigh accurately about 0.3 g of Nicorandil, dissolve in 30 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3), and titrate with 0.1 mol/L perchloric acid (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid
= 21.12 mg $C_8H_9N_3O_4$

Containers and Storage ***Containers*—**Tight containers. ***Storage*—**At a temperature between 2 °C and 8 °C.

Nicotinamide



$C_6H_6N_2O$: 122.13

Pyridine-3-carboxamide [*98-92-0*]

Nicotinamide, when dried, contains not less than 98.5 % and not more than 102.0 % of nicotinamide ($C_6H_6N_2O$).

Description Nicotinamide appears as white crystals or crystalline powder, is odorless and has a bitter taste. Nicotinamide is freely soluble in water or in ethanol (95) and slightly soluble in ether.

Identification (1) Mix 5 mg of Nicotinamide with 10 mg of 1-chloro-2,4-dinitrochlorobenzene, heat gently for 5 to 6 seconds and fuse the mixture. Cool and add 4 mL of potassium hydroxide-ethanol TS: a red color is observed.

(2) Take 20 mg of Nicotinamide, add 5 mL of sodium hydroxide TS and boil carefully: the gas evolved turns moistened red litmus paper blue.

(3) Determine the absorption spectra of solutions containing 20 mg of Nicotinamide and Nicotinamide RS in 1000 mL of water as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

pH Dissolve 1.0 g of Nicotinamide in 20 mL of water: the pH of this solution is between 6.0 and 7.5.

Melting Point 128 ~ 131 °C

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Nicotinamide in 20 mL of water: the solution is clear and colorless.

(2) ***Chloride***—Take 0.5 g of Nicotinamide and perform the test. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021 %).

(3) ***Sulfate***—Take 1.0 g of Nicotinamide and perform the test. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.019 %).

(4) ***Heavy metals***—Proceed with 1.0 g of Nicotinamide according to Method 1 and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

(5) ***Readily carbonizable substances***—Take 0.20 g of Nicotinamide and perform the test. The solution has no more color than Color Matching Fluid A.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, silica gel, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 25 mg each of Nicotinamide and Nicotinamide RS, previously dried, dissolve separately in 3 mL of water, and add the mobile phase to make them exactly 100 mL each. Pipet 8 mL each of these solutions, and add the mobile phase to make exactly 50 mL each. Pipet 5 mL each of these solutions, add exactly 5 mL each of the internal standard solution, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and calculate the ratios, Q_T and Q_S, of the peak area of nicotinamide to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of nicotinamide } (C_6H_6N_2O) = \text{Amount (mg) of Nicotinamide RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of nicotinic acid (1 in 25000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (particle diameter of 5 μm).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: Dissolve 1.0 g of sodium 1-heptane sulfonate in water to make 1000 mL. To 700 mL of this solution, add 300 mL of methanol.

Flow rate: Adjust the flow rate to make the retention time of nicotinamide being about 7 minutes.

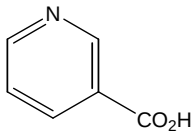
System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, nicotinic acid and nicotinamide are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nicotinamide to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Nicotinic Acid



$C_6H_5NO_2$: 123.11

Pyridine-3-carboxylic acid [*59-67-6*]

Nicotinic Acid, when dried, contains not less than 99.5 % and not more than 101.0 % of nicotinic acid ($C_6H_5NO_2$).

Description Nicotinic Acid appears as white crystals or crystalline powder, is odorless and has a slightly acid taste.

Nicotinic Acid is sparingly soluble in water, slightly soluble in ethanol (95) and very slightly soluble in ether.

Nicotinic Acid dissolves in sodium hydroxide TS or sodium carbonate TS.

Identification (1) Triturate 5 mg of Nicotinic Acid with 10 mg of 1-chloro-2,4-dinitrochlorobenzene and fuse the mixture by gentle heating for 5 to 6 seconds. Cool and add 4 mL of potassium hydroxide-ethanol TS: a dark red color is observed.

(2) Determine the absorption spectra of solutions containing 20 mg each of Nicotinic Acid and Nicotinic Acid RS in 1000 mL of water as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting Point 234 ~ 238 °C.

pH Dissolve 0.20 g of Nicotinic Acid in 20 mL of water: the pH of this solution is between 3.0 and 4.0.

Purity (1) ***Clarity and color of solution***—Dissolve 0.20 g of Nicotinic Acid in 20 mL of water: the solution is clear and colorless.

(2) ***Chloride***—Perform the test with 0.5 g of Nicotinic Acid. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021 %).

(3) ***Sulfate***—Dissolve 1.0 g of Nicotinic Acid in 3 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS and 3 mL of dilute hydrochloric acid and dilute with water to make 50 mL (not more than 0.019 %).

(4) ***Nitro compounds***—Dissolve 1.0 g of Nicotinic Acid in 8 mL of sodium hydroxide TS and add water to make 20 mL: the solution has no more color than Color Matching Fluid A.

(5) ***Heavy metals***—Proceed with 1.0 g of Nicotinic Acid according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 1 hour).

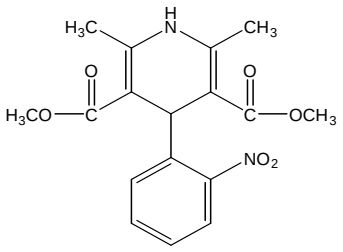
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.3 g of Nicotinic Acid, previously dried, dissolve in 50 mL of water and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 12.311 mg of $C_6H_5NO_2$

Containers and Storage ***Containers***—Well-closed containers.

Nifedipine



$C_{17}H_{18}N_2O_6$: 346.34

Dimethyl-2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate [*21829-25-4*]

Nifedipine, when dried, contains not less than 98.0 % and not more than 102.0 % of nifedipine ($C_{17}H_{18}N_2O_6$).

Description Nifedipine is a yellow, crystalline powder and is odorless and tasteless.
Nifedipine is freely soluble in acetone or in dichlormethane, sparingly soluble in methanol, in ethanol (95) or in acetic acid (100), slightly soluble in ether and practically insoluble in water.
Nifedipine is affected by light.

Identification (1) Dissolve 50 mg of Nifedipine in 5 mL of ethanol (95) and add 5 mL of hydrochloric acid and 2 g of zinc powder. Allow to stand for 5 minutes and filter. Perform the test with the filtrate as directed under the Qualitative Tests for primary aromatic amines: a red-purple color is observed.

(2) Determine the absorption spectra of solutions of Nifedipine and Nifedipine RS in methanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Nifedipine and Nifedipine RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both exhibit similar intensity of absorption at the same wavenumbers.

Melting Point 172 ~ 175 °C.

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Nifedipine in 5 mL of acetone: the solution is clear and yellow.

(2) ***Chloride***—Take 2.5 g of Nifedipine, add 12 mL of dilute acetic acid and 13 mL of water and heat to boil. After cooling, filter and discard the first 10 mL of the filtrate. To 5 mL of the subsequent filtrate, add 6 mL of dilute nitric acid and water to make 50 mL and perform the test. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021 %).

(3) ***Sulfate***—Take 4 mL of the filtrate obtained in (2) and add 1 mL of dilute hydrochloric acid and water

to make 50 mL. Perform the test. Prepare the control solution with 0.45 mL of 0.005 mol/L sulfuric acid VS (not more than 0.054 %).

(4) ***Heavy metals***—Proceed with 2.0 g of Nifedipine according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(5) ***Arsenic***—Prepare the test solution with 1.0 g Nifedipine according to Method 3 and perform the test (not more than 2 ppm).

(6) ***Basic substances***—Perform the test without exposure to light using light-resistant vessels. Dissolve 5.0 g of Nifedipine in 80 mL of a mixture of acetone and acetic acid (31) (5 : 3) and titrate with 0.02 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction: not more than 1.9 mL of 0.02 mol/L perchloric acid VS is consumed.

(7) ***Related substances***—Perform the test quickly without exposure to light using light-resistant vessels. Weigh accurately 25 mg of Nifedipine, dissolve in 25 mL of methanol, add the mobile phase to make exactly 250 mL and use this solution as the test solution. Separately, weigh precisely a suitable amount of Nifedipine RS and dissolve in methanol to render the concentration of 1 mg in 1 mL. Pipet accurately a portion of this solution, add mobile phase to render the concentration of 0.3 mg in 1 mL and use this solution as the standard solution for nifedipine. In addition, weigh a suitable portion of nifedipine nitrophenylpyridine analog RS, dissolve in methanol to render the concentration of the solution to be 1 mg in 1 mL. Pipet accurately a portion of this solution, add mobile phase to render the concentration of 0.6 mg in 1 mL and use this solution as the standard solution (1). Weigh a suitable portion of nifedipine nitrosophenylpyridine analog RS, dissolve in methanol to render the concentration of the solution to be 1 mg in 1 mL. Pipet accurately a portion of this solution, add mobile phase to render the concentration of 0.6 μg in 1 mL and use this solution as the standard solution (2). Transfer accurately 5 mL each the standard solution (1) and the standard solution (2) to a container, add exactly 5 mL the mobile phase to the container and use the solution as the standard solution for nifedipine related substances. Perform the test with 25 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Measure the peak areas of the nifedipine anlalogs in the test (A_T) and the standard (A_S) solution to determine the amount of the nifedipine analogs: the amounts for nifedipine nitrophenylpyridine analog and nifedipine nitrosophenylpyridine analog are not more than 0.2 %.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength 265 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μM in particle diameter). The assistant column contains octadecylsilanized silica gel.

Mobile phase: A mixture of water, methanol and acetonitrile (50:25:25)

Flow rate: 1 mL/minute

System suitability

System performance: When the procedure is run with 25 μL of the mixture of the standard solution for nifedipine and the standard solutions (1), (2) (1 : 1 : 1) under the above operating conditions, nifedipine nitrophenylpyridine, nifedipine nitrosophenylpyridine and nifedipine are eluted in this order. The resolution between the peak of nifedipine nitrophenylpyridine and nifedipine nitrosophenylpyridine is not less than 1.5, that between the peak of nitrosophenylpyridine and nifedipine is not less than 1.0.

System repeatability: When the test is repeated 6 times with 25 μL each of mixture according to above conditions, the relative standard deviation calculated on the peak area of each related compound is not more than 10 %.

Loss on Drying Not more than 0.5 % (0.5 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Perform the test protecting from the exposure to daylight, using light-resistant vessels. Weigh accurately about 0.12 g of Nifedipine and dissolve in methanol to make exactly 200 mL. Measure 5.0 mL of this solution and add methanol to make exactly 100 mL. Determine the absorbance of this solution, *A*, at the wavelength of a maximum absorption at about 350 nm, as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of nifedipine } (C_{17}H_{18}N_2O_6) = \frac{A}{142.3}\times 40000$$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Nifedipine Capsules

Nifedipine Capsules contains not less than 90.0 % and not more than 110.0 % of labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$: 346.34).

Method of Preparation Prepare as directed under Capsules, with Nifedipine.

Identification (1) Transfer the contents of 3 Nifedipine Capsules to a centrifuge tube, add 20 mL of 0.1 mol/L sodium hydroxide TS and 25 mL of dichloromethane. Insert a stopper, invert several times,

and carefully release the pressure in the tube. Insert the stopper again, and shake gently for 1 hour. Centrifuge the tube for 10 minutes at 2000 to 2500 revolution per minute, use the clarified lower layer as the test solution. Separately, transfer a portion of Nifedipine RS, dissolve in dichloromethane to contain 1.2 mg per mL, and use this solution as the standard solution. Mix equal volumes of the standard solution and the test solution, use this solution as the mixture. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 500 μL each of the test solution, the standard solution and the mixture on a plate with a 0.5 mm layer of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and cyclohexane (1 : 1), to a distance of about 15 cm and air-dry the plate. Immediately examine the plate under ultraviolet light (main wavelength 254 nm): each solution exhibits a dark blue major band at a same R_f value of about 0.3. Spray the plate with the visualizing solution: each solution exhibits a compact light orange band on a yellow background.

(2) The retention time of the principal peak in the chromatogram of the test solution corresponds to that in the chromatogram of the standard solution, as obtained in the Assay.

Visualizing solution—Dissolve 3 g of bismuth subnitrate and 30 g of potassium iodide with 10 mL of 3 mol/L hydrochloric acid and add water to make 100 mL. Prior to use, transfer exactly 10 mL of solution, add 10 mL of 3 mol/L hydrochloric acid and water to make 100 mL.

Related Substances Perform this procedure quickly without exposure to light using light-resistant vessels. Prepare the test solution as direded in the test solution under Assay. Separately, weigh accurately suitable amount of Nifedipine RS in methanol to make contain 1 mg per mL. Pipet suitable amount of this solution, add the mobile phase to make contain 0.3 mg per mL and use this solution as the standard solution for nifedipine. Weigh accurately nifedipine nitrophenylpyridine analog RS, dissolve in methanol to make the concentration of 1 mg per mL. Pipet suitable amount of this solution, add the mobile phase to render the concentration of 6 μg per mL and use this solution as the standard solution (1). Weigh accurately nifedipine nitrosophenylpyridine analog RS, dissolve in methanol to make the concentration of 1 mg per mL. Pipet suitable amount of this solution, add the mobile phase to render the concentration of 1.5 μg per mL and use this solution as the standard solution (2). Pipet 5.0 mL each of the standard solution (1) and (2), add 5.0 mL of the mobile phase. Use this solution as the standard solution for nifedipine related substances. Perform the test with 25 μL each of the test solution and the standard solutions as directed under Liquid Chromatography according to the following operating conditions. Determine each peak area, A_T and A_S, of nifedipine related substances from each solution, calculate the amounts of nifedipine related compounds. 4-(2-nitropheny)-2,6-dimethylpyrine-3,5-dicarboxylic acid, corresponding to nifedipine nitrophenylpyridine, is not more than 2.0 % and dimethyl 4-(2-nitroso phenyl)-2,6-dimethylpyridine-3,5-dicarboxylic acid, corresponding to nifedipine nitrosophenylpyridine, is not more than 0.5 %.

Operating conditions

Detector, Column, Mobile phase, Flow rate: Proceed as directed in the operating conditions under the Assay

System suitability

System performance: When the procedure is run with 25 μL of the mixture of the standard solution for nifedipine and the standard solutions (1), (2) (1 : 1 : 1) under the above operating conditions, nifedipine nitrophenylpyridine, nifedipine nitrosophenylpyridine and nifedipine are eluted in this order. The resolution between the peak of nifedipine nitrophenylpyridine and nifedipine nitrosophenylpyridine is not less than 1.5, that between the peak of nitrosophenylpyridine and nifedipine is not less than 1.0.

System repeatability: When the test is repeated 6 times with 25 μL each of mixture according to above conditions, the relative standard deviation calculated on the peak area of each related compound is not more than 10 %.

Dissolution Test Perform the test with 1 capsule of Nifedipine Capsules at 50 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of the first solution in Disintegration as the dissolution solution. Take the dissolved solution 20 minutes after starting the test, filter, and use the filtrate as the test solution for the Assay. If necessary, suitably dilute with the dissolution solution. Separately, weigh accurately a portion of Nifedipine RS, add the dissolution solution to make the same concentration with the test solution, and use this solution as the standard solution. Determine the ultraviolet absorbance at the wave length of a maximum absorbance at about 340 nm of the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry using the dissolution solution as the blank.

The dissolution rate of Nifedipine Capsules in 20 minutes is not less than 80 %.

Uniformity of Dosage Units It meets the requirement.

Assay Perform this procedure quickly without exposure to light using light-resistant vessels. Transfer the contents of 5 capsules with the aid of a small amount of methanol to a volumetric flask, quantitatively dilute with mobile phase to obtain a total volume, V mL, of solution having a concentration of about 0.1 mg of nifedipine per mL. Separately, take accurately suitable amount of Nifedipine RS, dissolve in methanol to make contain 1 mg per mL. Pipet suitable amount of

this solution, add the mobile phase to make contain 0.1 mg per mL. Use this solution as the standard solution. Perform the test with 25 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine each peak area, A_T and A_S, of Nifedipine for the test solution and the standard solution.

$$\text{Amount (mg) of nifedipine } (C_{17}H_{18}N_2O_6) \text{ in 1 capsule} = \frac{VC}{5} \times \frac{A_T}{A_S}$$

C: Concentration (mg/mL) of Nifedipine RS in the standard solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column, 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilyl silica gel for liquid chromatography (5 μm in particle diameter). The assistant column contains octadecylsilyl silica gels.

Mobile phase: A mixture of water, methanol and acetonitrile (50 : 25 : 25).

Flow rate: 1 mL/minute.

System suitability

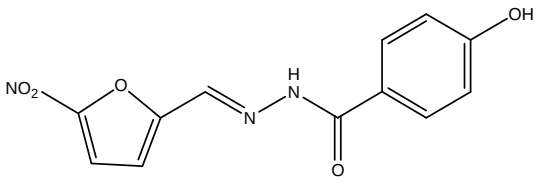
System performance: When the procedure is run with 25 μL of the standard solution according to the above operating conditions, the symmetry factor is not more than 1.5.

System repeatability: When the test is repeated 6 times with 25 μL each of the standard solution according the above operating conditions, the relative standard deviation calculated on the peak area of nifedipine is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and at a temperature between 15ºC and 25ºC.

Nifuroxazide



$C_{12}H_9N_3O_5$: 275.22

(*E*)-4-Hydroxy-*N*′-((5-nitrofuran-2-yl)methylene) benzohydrazide [*965-52-6*]

Nifuroxazide, when dried, contains not less than 98.5 % and not more than 101.5 % of nifuroxazide ($C_{12}H_9N_3O_5$).

Description Nifuroxazide is a bright yellow crystalline powder.

Nifuroxazide is slightly soluble in ethanol (95) or in dichloromethane, and practically insoluble in water.

Identification Determine the infrared spectra of Nifuroxazide and Nifuroxazide RS, both previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Absorbance $E_{1\,cm}^{1\%}$ (367 nm): 940 ~ 1000. Under the protection of light, weigh accurately 10.0 mg of Nifuroxazide, add 10 mL of ethylene glycol monomethyl ether and methanol to make exactly 100 mL. Pipet 5.0 mL of the solution and add methanol to make 100 mL.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Nifuroxazide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substance I***—Dissolve 1 g of Nifuroxazide in 10 mL of dimethylsulfoxide and use this solution as the test solution (1). To 5.5 mL of the test solution (1), add 50.0 mL of water while stirring, allow the solution to stand for 15 minutes, filter and use the filtrate as the test solution (2). To 0.5 mL of the test solution (1), dissolve 50 mg of nifuroxazide related substance I (4-hydroxybenzohydroxazide) and add dimethylsulfoxide to make 1000 mL. To 5 mL of this solution, add 50.0 mL of water while stirring, allow the solution to stand for 15 minutes, filter and use the filtrate as the standard solution. To 10 mL each of the test solution (2) and the standard solution, add 0.5 mL each of phosphomolybdotungstic acid TS and 10.0 mL each of 2 mol/L sodium carbonate TS and allow the mixture to stand for 1 hour. Determine the absorbances of the test solution (2) and the standard solution at 750 nm as directed under Ultraviolet-visible Spectrophotometry: the absorbance obtained from the test solution (2) is not greater than that from the standard solution (not more than 0.05 %).

(3) ***Other related substances***—Weigh accurately 0.1 g of Nifuroxazide, dissolve in dimethylsulfoxide, add the mobile phase to make exactly 100 mL and use this solution as the test solution. Weigh accurately 10 mg of nifuroxazide related substance II (methyl parahydroxybenzoate), dissolve in 2 mL of *N,N*-dimethylformamide and add the mobile phase to make 20 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL and use this solution as the standard solution (1). Weigh accurately 5 mg of Nifuroxazide and 10 mg of nifuroxazide related substance II, dissolve in 2 mL of *N,N*-dimethylformamide and add the mobile phase to make 20 mL. Pipet 1 mL

of this solution, add the mobile phase to make exactly 100 mL and use this solution as the standard solution (2). Protect the test solution, the standard solution (1) and (2) from light after the preparation and perform the test with 20 μL each of these solutions as directed under Liquid Chromatography: the number of peak having 0.6 times the area of the principal peak obtained from the standard solution (1) is not more than 1 from the test solution (0.3 %), the area of such peak from the test solution is greater than 0.2 times the area of the principal peak obtained from the standard solution (1) (0.1 %) and total area of the peaks of the related substances from the test solution is not larger than the area of the principal peak from the standard solution (1) (0.5 %). Disregard any peak having the area not more than 0.1 times the area of the principal peak obtained from the standard solution (1) (0.05 %).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of water and acetonitrile (65:35)

Flow rate: 1.0 mL/minute.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution (2) under the above operating conditions, the resolution between the nifuroxazide and the related substance II is not less than 4. With the reference retention time of nifuroxazide of 6.5 minute, the retention time of the related substances I, II, III and IV is about 0.4, 1.2, 2.8 and 5.2 minutes, respectively.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

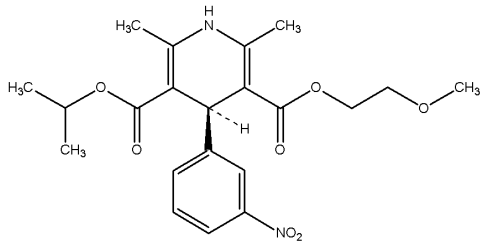
Assay Weigh accurately about 0.2 g of Nifuroxazide, previously dried, dissolve in 30 mL of *N,N*-dimethylformamide, heat, if necessary, to dissolve, add 20 mL of water and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 27.52 mg of $C_{12}H_9N_3O_5$

Containers and Storage ***Containers***—Tight containers

Storage—Light-resistant.

Nimodipine



and enantiomer

$C_{21}H_{26}N_2O_7$: 418.44

(*RS*)-3-(2-Methoxyethyl) 5-propan-2-yl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate [*66085-59-4*]

Nimodipine contains not less than 98.5 % and not more than 101.5 % of nimodipine ($C_{21}H_{26}N_2O_7$), calculated on the dried basis.

Description Nimodipine is a light yellow or yellow crystalline powder.

Nimodipine is freely soluble in ethyl acetate, sparingly soluble in ethanol (95) and practically insoluble in water.

Nimodipine is affected by light.

Nimodipine shows crystalline polymorphism.

Identification (1) Determine the infrared spectra of Nimodipine and Nimodipine RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) When proceed as directed in the Assay, the retention time of the principal peak from the test solution is same as that from the standard solution.

Specific Optical Rotation $[\alpha]_D^{20}$: -10 ~ +10° (1g, acetone, 20 mL, 100 mm).

Purity ***Related substances***—Weigh accurately about 40 mg of Nimodipine, dissolve in 2.5 mL of tetrahydrofuran, add the mobile phase to make exactly 25 mL and use this solution as the test solution. Separately, weigh accurately about 40 mg of Nimodipine RS, dissolve in 2.5 mL of tetrahydrofuran, add the mobile phase to make exactly 25 mL, pipet 1.0 mL of this solution, add the mobile phase to make 100 mL, pipet 2.0 mL of this solution, add the mobile phase to make exactly 10 mL and use this solution as the standard solution (1). Weigh accurately about 20 mg of nimodipine related substance I [2-methoxyethyl 1-methylethyl 2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate], dissolve in 2.5 mL of tetrahydrofuran, add the mobile phase to make exactly 25 mL, pipet 5.0 mL of this solution, add the mobile phase to make exactly 100 mL and use this solution as

the standard solution (2). Pipet 2.5 mL of the standard solution (1), add the mobile phase to make exactly 100 mL and use this solution as the standard solution (3). Pipet 1.0 mL each of the standard solution (2) and the standard solution (3), transfer to a 25 mL volumatic flask, add the mobile phase to make exactly 25 mL and use this solution as the standard solution (4). Perform the test with 20 μL each of the test solution, the standard solution (1) and the standard solution (4) as directed under Liquid Chromatography according the following operating conditions and determine the area of the peak of each related substance in the test solution, A_T, and the area of the peak of the related substance I in the standard solution (4), A_S: the related substance I is not more than 0.1 %, each related substance other than the related substance I is not more than 0.2 %, and the total related substances is not more than 0.5 %.

$$\text{Content (\%) of each related substance} = 100 \times C \times \frac{A_T}{A_S}$$

C: Concentration (mg/mL) of nimodipine related substance I in the standard solution (4).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 12.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm to 10 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: A mixture of water, methanol and tetrahydrofuran (3:1:1).

Flow rate: 2 mL/minute.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution (4) under the above operating conditions, the relative retention time for the peak of the related substance I and of nimodipine is 0.9 and 1.0, respectively, with the resolution between these two peaks being not less than 1.5.

System repeatability: When the test is repeated 5 times with 20 μL each of the standard solution (4) under the above operating conditions, the relative standard deviation of the peak area of nimodipine is not more than 2.0 %.

Loss on Drying Not more than 0.5 % (1.0 g, 105 °C, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

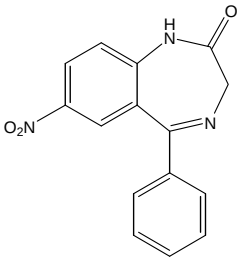
Assay Weigh accurately about 0.180 g of Nimodipine, add to a mixture of 25 mL of *t*-butyl alcohol and 25 mL perchloric acid TS and heat gently to dissolve. Cool and titrate with 0.1 mol/L cerium sulfate VS (indicator: 0.1 mL of ferroin TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L cerium sulfate VS
= 20.92 mg of $C_{21}H_{26}N_2O_7$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and between 15 °C and 30 °C.

Nitrazepam



$C_{15}H_{11}N_3O_3$: 281.27

7-Nitro-5-phenyl-1*H*-benzo[e][1,4]diazepin-2(3*H*)-one [*146-22-5*]

Nitrazepam, when dried, contains not less than 99.0 % and not more than 101.0 % of nitrazepam ($C_{15}H_{11}N_3O_3$).

Description Nitrazepam appears as white to pale yellow crystals or crystalline powder and is odorless.
Nitrazepam is freely soluble in acetic acid (100), soluble in acetone or in chloroform, slightly soluble in methanol, in ethanol or in dehydrated ethanol, very slightly soluble in ether and practically insoluble in water.

Melting point—About 227 °C (with decomposition).

Identification (1) Take 3 mL of a solution of Nitrazepam in methanol (1 in 500) and add 0.1 mL of sodium hydroxide TS: a yellow color is observed.

(2) Take 20 mg of Nitrazepam, add 15 mL of dilute hydrochloric acid, boil for 5 minutes, cool and filter: the filtrate responds to the Qualitative Tests for primary aromatic amines.

(3) Neutralize 0.5 mL of the filtrate obtained in (2) with sodium hydroxide TS, add 2 mL of ninhydrin TS and heat on a water-bath: a purple color is observed.

(4) Determine the absorption spectra of solutions of Nitrazepam and Nitrazepam RS in dehydrated ethanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) ***Clarity and color of solution***—Dissolve 0.10 g of Nitrazepam in 20 mL of acetone: the solution is clear and pale yellow in color.

(2) ***Heavy metals***—Proceed with 1.0 g of Nitrazepam according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Nitrazepam according to Method 3 and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 0.25 g of Nitrazepam in 10 mL of a mixture of methanol and chloroform (1 : 1) and use this solution as the test solution. Pipet 1.0 mL of the test solution, add a mixture of methanol and chloroform (1 : 1) to make exactly 20 mL. Pipet 2.0 mL of this solution, add a mixture of methanol and chloroform (1 : 1) to make exactly 50 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of nitromethane and ethyl acetate (17 : 3) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

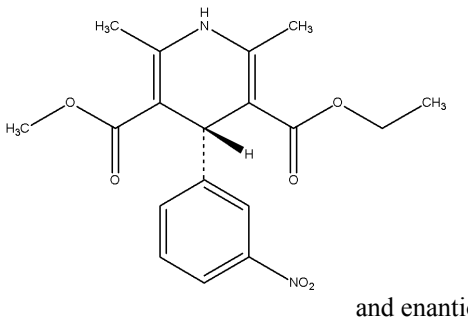
Assay Weigh accurately about 0.4 g of Nitrazepam, previously dried and dissolve in 40 mL of acetic acid (100). Titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 28.127 mg of $C_{15}H_{11}N_3O_3$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Nitrendipine



and enantiomer

$C_{18}H_{20}N_2O_6$: 360.36

Ethyl methyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydro pyridine-3,5-dicarboxylate [*39562-70-4*]

Nitrendipine, when dried, contains not less than 98.5 % and not more than 101.0 % of nitrendipine ($C_{18}H_{20}N_2O_6$).

Description Nitrendipine is a yellow crystalline powder.
Nitrendipine is soluble in acetonitrile, sparingly soluble in methanol or in ethanol (99.5) and practically insoluble in water.
Nitrendipine is gradually colored to brownish yellow by light.
A solution of Nitrendipine in acetonitrile (1 in 50) shows no optical rotation.

Identification (1) Determine the absorption spectra of solutions of Nitrendipine and Nitrendipine RS in methanol (1 in 80000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Nitrendipine and Nitrendipine RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 157 ~ 161 °C.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Nitrendipine according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Perform the test quickly without exposure to light using light-resistant vessels. Dissolve 40.0 mg of Nitrendipine in 5 mL of acetonitrile, add the mobile phase to make exactly 25 mL and use this solution as the test solution. Pipet 1.0 mL of the test solution, add the mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the area of the peak other than the principal peak in the test solution, A_I, and the area of the nitrendipine peak in the standard solution, A_S, by the automatic integration method: the related substance having the retention time relative to nitrendipine of about 0.8 is not more than 1.0 %, the related substance having the retention time relative to nitrendipine of about 1.3 is not more than 0.25 %, the individual amount of any other related substances is not more than 0.2 %, and the total amount of related substances other than nitrendipine is not more than 2.0 %.

Content (%) of each related substance = $\frac{A_i}{A_S}$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: A mixture of water, tetrahydrofuran and acetonitrile (14:6:5).

Flow rate: Adjust the flow rate so that the retention time of nitrendipine is about 12 minutes.

System suitability

Test for required detectability: Pipet accurately 2 mL of the standard solution and add the mobile phase to make exactly 10 mL. Confirm that the peak area of nitredipine from 10 μL of this solution is equivalent to between 14 % and 26 % of that from the standard solution.

System performance: Dissolve 10 mg of Nitrendipine and 3 mg propyl parahydroxybenzoate in 5 mL of acetonitrile and add the mobile phase to make 100 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, propyl parahydroxybenzoate and nitrendipine are eluted in this order with the resolution between their peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nitrendipine is not more than 2.0 %.

Time span of measurement: About 2.5 times as long as the retention time of nitrendipine beginning after the solvent peak.

Loss on Drying Not more than 0.5 % (1.0 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.3 g of Nitrendipine, previously dried, add 60 mL of sulfuric acid in ethanol (99.5) (3 in 100), add 50 mL of water and titrate with 0.1 mol/L ceric ammonium sulfate VS (indicator: 3 drops of 1,10-phenanthroline hydrate TS) until the brownish-red color of the solution disappears. Perform a blank determination and make any necessary correction.

Each mL of ceric ammonium sulfate VS
= 18.02 mg of $C_{18}H_{20}N_2O_6$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Nitroglycerin Tablets

Nitroglycerin Tablets contain not less than 80.0 % and not more than 120.0 % of the labeled amount of nitroglycerin ($C_3H_5N_3O_9$: 227.09).

Method of Preparation Prepare as directed under the Tablets, with nitroglycerin.

Identification (1) Weigh a portion of powdered Nitroglycerin Tablets, equivalent to 6 mg of nitroglycerin ($C_3H_5N_3O_9$) according to the labeled amount, shake thoroughly with 12 mL of ether, filter and use the filtrate as the test solution. Evaporate 5 mL of the test solution, dissolve the residue in 1 to 2 drops of sulfuric acid and add 1 drop of diphenylamine TS: a deep blue color is observed.

(2) Evaporate 5 mL of the test solution obtained in (1), add 5 drops of sodium hydroxide TS, heat over a low flame and concentrate to about 0.1 mL. Cool and heat the residue with 20 mg of potassium bisulfate: the odor of acrolein is perceptible.

Purity ***Free nitrate ion***—Transfer an accurately measured portion of powdered Nitroglycerin Tablets, equivalent to 20 mg of nitroglycerin ($C_3H_5N_3O_9$) according to the labeled amount, to a separatory funnel, add 40 mL of isopropylether and 40 mL of water, shake for 10 minutes, collect the aqueous layer, filter and use the filtrate as the test solution. Separately, transfer 10 mL of standard nitric acid solution to a separatory funnel, add 30 mL of water and 40 mL of the isopropyl ether layer of the first extraction of the test solution, shake for 10 minutes, continue the procedure in the same manner as the test solution and use this solution as the standard solution. Transfer 20 mL each of the test solution and the standard solution to Nessler tubes, shake well with 30 mL of water and 60 mg of Griess-Romijin's nitric acid reagent, allow to stand for 30 minutes and observe the tubes horizontally: the test solution has no more color than the standard solution.

Disintegration Test It meets the requirement, provided that the time limit of the test is 2 minutes and the use of the auxiliary disks is omitted.

Uniformity of Dosage Units It meets the requirement when the content uniformity test is performed according to the following procedure.

Transfer 1 tablet of Nitroglycerin Tablets to a glass-stoppered centrifuge tube and add exactly *V* mL of acetic acid (100) to make a solution containing about 30 μg of nitroglycerin ($C_3H_5N_3O_9$) per mL. Shake vigorously for 1 hour and after disintegrating the tablet, centrifuge and use the clear supernatant liquid as the test solution. When the tablet does not disintegrate during this procedure, transfer 1 tablet of Nitroglycerin Tablets to a glass-stoppered centrifuge tube, wet the tablet

with 0.05 mL of acetic acid (100) and grind with a glass rod. While rinsing the glass rod, add acetic acid (100) to make exactly V mL of a solution containing about 30 μg of nitroglycerin ($C_3H_5N_3O_9$) per mL. Shake for 1 hour, centrifuge and use the clear supernatant liquid as the test solution. Separately, weigh accurately about 90 mg of potassium nitrate, previously dried at 105 °C for 4 hours, dissolve in 5 mL of water and add acetic acid (100) to make exactly 100 mL. Pipet 5.0 mL of the solution, add acetic acid (100) to make exactly 100 mL and use this solution as the standard solution. Measure exactly 2 mL each of the test solution and the standard solution, add 2.0 mL each of salicylic acid TS, shake, allow to stand for 15 minutes and add 10 mL each of water, cooling with ice. Render the solution alkaline with about 12 mL of a solution of sodium hydroxide (2 in 5) while cooling in ice and add water to make exactly 50 mL. Perform the test with the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry, using a solution prepared with 2 mL of acetic acid (100) in the same manner, as the blank. Determine the absorbances, A_T and A_S, of the subsequent solutions of the test solution and the standard solution at 410 nm, respectively.

$$\text{Amount (mg) of nitroglycerin } (C_3H_5N_3O_9) = \text{Amount (mg) of Potassium nitrate RS} \times 0.7487 \times \frac{A_T}{A_S} \times \frac{V}{2000}$$

Calculate the average content from the contents of 10 Nitroglycerin Tablets: it meets the requirement when each content deviation (%) from the average content is not exceeding 25 %. When there is 1 tablet showing a deviation exceeding 25 % and not exceeding 30 %, perform the test with additional 20 Nitroglycerin Tablets in the same manner. Calculate the average content of all 30 tablets and the deviations (%) from the new average: it meets the requirement when no more than 1 tablet shows a deviation exceeding 25 % and not exceeding 30 %, but no tablet deviates by more than 30 %.

Assay Weigh accurately and disintegrate, by soft pressing, not less than 20 Nitroglycerin Tablets. Weigh accurately a portion of the powder, equivalent to about 3.5 mg of nitroglycerin ($C_3H_5N_3O_9$), add exactly 50 mL of acetic acid (100), shake for 1 hour, filter and use this filtrate as the test solution. Separately, weigh accurately about 90 mg of potassium nitrate, previously dried at 105 °C for 4 hours, dissolve in 5 mL of water and add acetic acid (100) to make exactly 100 mL. Pipet 10.0 mL of the solution, add acetic acid (100) to make exactly 100 mL and use this solution as the standard solution. To 2.0 mL each of the test solution and the standard solution, add 2 mL of salicylic acid TS, shake, allow to stand for 15 minutes and add 10 mL of water, cooling with ice. Render the solution alkaline with about 12 mL of a solution of sodium hydroxide (2 in 5) while cooling in ice and add water to make exactly 50 mL. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry, using a solution, prepared with 2 mL of acetic acid (100) in the same manner, as the blank. Determine the absorbances, A_T and A_S, of the subsequent solutions of the test solution and the standard solution at 410 nm, respectively.

$$\text{Amount (mg) of nitroglycerin } (C_3H_5N_3O_9) = \text{Amount (mg) of potassium nitrate} \times 0.7487 \times \frac{A_T}{A_S} \times \frac{1}{20}$$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and not exceeding 20 °C.

Nitrous Oxide

N_2O: 44.01

Nitrous oxide [*10024-97-2*]

Nitrous Oxide contains not less than 97.0 vol % and not more than 101.0 vol % of nitrous oxide (N_2O).

Description Nitrous Oxide is a colorless gas at room temperature and at atmospheric pressure and is odorless.

Nitrous Oxide is soluble in ether or in fatty oils.

1 mL of Nitrous Oxide dissolves in 1.5 mL of water or 0.4 mL of ethanol (95) at 20 °C and at 101.3 kPa.

1000 mL of Nitrous Oxide at 0 °C and at 101.3 kPa weighs about 1.96 g.

Identification (1) A glowing splinter of wood held in Nitrous Oxide: it bursts into flame immediately.

(2) Transfer 1 mL each of Nitrous Oxide and nitrous oxide directly from metal cylinders with a pressure-reducing valve to gas measuring tubes or syringes for gas chromatography, using a polyvinyl chloride induction tube. Perform the test with these gases as directed under Gas Chromatography according to the operating conditions under the Assay: the retention time of the main peak from Nitrous Oxide corresponds with that of nitrous oxide.

Purity Maintain the containers of Nitrous Oxide between 18 °C and 22 °C for more than 6 hours before the test and correct the volume at 20 °C and at 101.3 kPa.

(1) ***Acid or alkali***—Take 400 mL of freshly boiled and cooled water, add 0.3 mL of methyl red TS and 0.3 mL of bromthymol blue TS and boil for 5 minutes. Transfer 50 mL of this solution to each of three Nessler tubes marked A, B and C. Add 0.10 mL of 0.01 mol/L hydrochloric acid VS to tube A, 0.20 mL of 0.01 mol/L hydrochloric acid VS to tube B, stopper each of the

tubes and cool. Pass 1000 mL of Nitrous Oxide through the solution in tube A for 15 minutes, employing delivery tube with an orifice approximately 1 mm in diameter and extending to within 2 mm of the bottom of the Nessler tube: the color of the solution in tube A is not deeper orange-red than that of the solution in tube B and not deeper yellow-green than that of the solution in tube C.

(2) ***Carbon dioxide***—Pass 1000 mL of Nitrous Oxide through 50 mL of barium hydroxide TS in a Nessler tube, in the same manner as directed in (1): any turbidity produced does not exceed that produced in the following control solution.

Control solution—Take 50 mL of barium hydroxide TS in a Nessler tube, add 1 mL of a solution of 0.1 g of sodium bicarbonate in 100 mL of freshly boiled and cooled water.

(3) ***Oxidizing substances***—Transfer 15 mL of potassium iodide-starch TS to each of two Nessler tubes marked A and B, add 1 drop of acetic acid (100) to each of the tubes, shake and use these solutions as solution A and solution B, respectively. Pass 2 L of Nitrous Oxide through solution A for 30 minutes in the same manner as directed in (1): the color of solution A is the same as that of the stoppered, untreated solution B.

(4) ***Potassium permanganate and reducing substance***—Pour 50 mL of water into each of two Nessler tubes marked A and B, add 0.10 mL of 0.02 mol/L potassium permanganate VS to each of the tubes and use these solutions as solutions A and B, respectively. Pass 1000 mL of Nitrous Oxide through solution A in the manner as directed in (1): the color of solution A is the same as that of solution B.

(5) ***Chloride***—Pour 50 mL of water into each of two Nessler tubes marked A and B, add 0.5 mL of silver nitrate TS to each of the tubes, shake and use these solutions as solutions A and B, respectively. Pass 1000 mL of Nitrous Oxide through solution A in the same manner as directed in (1): the turbidity of solution A is the same as that of solution B.

(6) ***Carbon monoxide***—Introduce 5.0 mL of Nitrous Oxide into a gas-cylinder or a syringe for gas chromatography from a metal cylinder holding gas under pressure and fitted with a pressure-reducing valve, through a directly connected polyvinyl tube. Perform the test according to the Gas Chromatography under the following conditions: no peak is observed at the same retention time as that of carbon monoxide.

Operating conditions

Detector: A thermal conductivity detector.

Column: A column, about 3 mm in internal diameter and about 3 m in length, packed with 300 μm to 500 μm zeolite for gas chromatography (0.5 nm in pore size).

Column temperature: A constant temperature of about 50 °C.

Carrier gas: Hydrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of carbon monoxide is about 20 minutes.

System suitability

Detection sensitivity: Adjust the sensitivity so that the peak height of carbon monoxide obtained from 5.0 mL of the mixed gas used in the system performance is about 10 cm.

System performance: Take 0.1 mL each of carbon monoxide and air in a gas mixer, add carrier gas to make 100 mL and mix well. When the procedure is run with 5.0 mL of the mixed gas under the above operating conditions, oxygen, nitrogen and carbon monoxide are eluted in this order with well resolved peaks.

(7) ***Nitric oxide***—Proceed in the same manner as directed in the Purity (8) Nitrogen dioxide. Pass 500 ± 50 mL of Nitrous Oxide in the gas phase through a nitric oxide-nitrogen dioxide detector tube at the specified rate, and determine the amount of nitric oxide: not more than 1 ppm.

(8) ***Nitrogen dioxide***—To a cylinder, connect a piece of tubing of sufficient length to allow all the contents in the liquid phase to vaporize during passage through it when the valve is opened, and prevent frost from reaching the inlet of the detector tube. Pass 550 ± 50 mL of Nitrous Oxide in the gas phase through a nitric oxide-nitrogen dioxide detector tube at the specified rate via the tubing (previously flush the system with Nitrous Oxide to displace air), and determine the amount of nitrogen dioxide: not more than 1 ppm. Measure the gases with a gas volume meter downstream from the detector tube in order to prevent contamination.

Assay Take Nitrous Oxide as directed in the Purity. Introduce 1.0 mL of Nitrous Oxide into a gas-measuring tube or syringe for gas chromatography from a metal cylinder under pressure through a pressure-reducing valve and a directly connected polyvinyl tube. Perform the test with this gas as directed under Gas Chromatography according to the following operating conditions and determine the peak area, A_T, of nitrogen. Separately, introduce 3.0 mL of nitrogen into a gas mixer, add carrier gas to make exactly 100 mL, mix thoroughly and use this gas as the standard mixed gas. Proceed with 1.0 mL of this mixture in the same manner and determine the peak area, A_S, of nitrogen.

$$\text{Amount (vol \%) of nitrous oxide } (N_2O) = 100 - 3 \times \frac{A_T}{A_S}$$

Operating conditions

Detector: A thermal conductivity detector.

Column: A column, about 3 mm in internal diameter and about 3 m in length, packed with silica gel for gas chromatography (300 μm to 500 μm in particle diameter).

Column temperature: A constant temperature of

about 50 °C.

Carrier gas: Hydrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of nitrogen is about 2 minutes.

System suitability

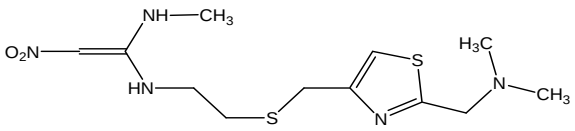
System performance: Take 3.0 mL of nitrogen in a gas mixer, add nitrous oxide to make 100 mL and mix well. When the procedure is run with 1.0 mL of the mixed gas under the above operating conditions, nitrogen and nitrous oxide are eluted in this order with well resolved peaks.

System repeatability: When the test is repeated 6 times with the standard mixed gas under the above operating conditions, the relative standard deviation of the peak area of nitrogen is not more than 2.0 %.

Containers and Storage ***Containers***—Metal cylinders hermetic containers.

Storage—Not exceeding 40 °C.

Nizatidine



$C_{12}H_{21}N_6O_2S_2$: 331.46

(*E*)-1-*N*'-[2-[[2-[(Dimethylamino)methyl]-1,3-thiazol-4-yl]methylsulfanyl]ethyl]-1-*N*-methyl-2-nitroethene-1,1-diamine [*76963-41-2*]

Nizatidine contains not less than 98.0 % and not more than 101.0 % of nizatidine ($C_{12}H_{21}N_6O_2S_2$), calculated on the anhydrous basis.

Description Nizatidine appears as white or pale brown, crystalline powder.

Nizatidine is freely soluble in acetic acid (100), soluble in methanol, sparingly soluble in water, slightly soluble in ethanol (99.5), in 2-propanol or in acetic anhydride and practically insoluble in ether.

Identification (1) Determine the infrared spectra of Nizatidine and Nizatidine RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) When proceed as directed in the Assay, the retention time of the principal peak from the test solution corresponds to that from the standard solution.

Melting Point 130 ~ 135 °C. Perform the test after drying.

Purity (1) ***Heavy metals***—Proceed with 0.1 g of Nizatidine according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Weigh accurately about 50 mg of Nizatidine, add a mixture of ammonium acetate buffer and methanol (76 : 24) to make exactly 10 mL and use this solution as the test solution. Separately, weigh accurately about 50 mg of Nizatidine RS, add a mixture of ammonium acetate buffer and methanol (76 : 24) to make exactly 100 mL, pipet 5.0 mL of this solution, add a mixture of ammonium acetate buffer and methanol (76 : 24) to make exactly 50 mL and use this solution as the standard solution (1). Pipet two portions of the standard solution (1), dilute each with a mixture of ammonium acetate buffer and methanol (76 : 24) to render the concentrations of 25 μg per mL and 15 μg per mL, respectively, and use these solutions as the standard solution (2) and the standard solution (3), respectively. Perform the test with 50 μL each of the test solution, the standard solutions (1), (2) and (3) as directed in the peak area percentage method under Liquid Chromatography according to the following operating condition: total area of the peaks other than the principal peak obtained from the test solution is not more than 3 times the area of the principal peak obtained from the standard solution (2) (1.5 %) and the area of each peaks other than the principal peak from the test solution is not more than the area of the principal peak from the standard solution (3) (0.3 %).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: Ammonium acetate buffer

Mobile phase B: Methanol

Adjust the composition of the mobile phase so that the retention time of nizatidine is about 12 minutes.

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-3	76	24
3-20	76→50	24→50
20-45	50	50
45-50	50→76	50→24
50-70	76	24

Flow rate: 1 mL/minute.

System suitability

System performance: When the procedure is run with 50 μL of the standard solution (1) under the above operating conditions, the symmetry factor for the nizatidine peak is not more than 2.0.

Time span of measurement: About 3 times as long as the retention time of nizatidine peak.

Ammonium acetate buffer—Weigh 5.9 g of ammonium acetate, dissolve in 760 mL of water to render the concentration of the solution to be 0.1 mol/L, add 1 mL of dimethylamine and adjust the pH to 7.5 with acetic acid.

Loss on Drying Not more than 1.0 % (2 g, 100 °C, 1 hour).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 15 mg of Nizatidine, add the mobile phase to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately about 15 mg of Nizatidine RS, add the mobile phase to make exactly 50 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the area of nizatidine peak in the test solution, A_T, and in the standard solution, A_S.

$$\text{Amount (mg) of nizatidine } (C_{12}H_{21}N_6O_2S_2) = \text{Amount (mg) of Nizatidine RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of ammonium acetate buffer and methanol (76:24).

Flow rate: 1 mL/minute.

System suitability

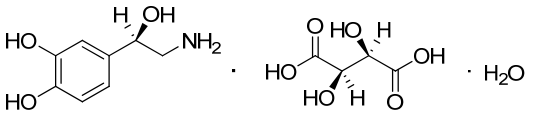
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates is not less than 1500 with the symmetry factor being not more than 2.0.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nizatidine is not more than 1.5 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Norepinephrine Tartrate Hydrate



and enantiomer

Noradrenaline Tartrate
Norepirenamine Tartrate

$C_8H_{11}NO_3 \cdot C_4H_6O_6 \cdot H_2O$: 337.28

(*RS*)-4-(2-Amino-1-hydroxyethyl)benzene-1,2-diol; (*RR*)- & (*SS*)-2,3-dihydroxybutanedioic acid; hydrate [*69815-49-2*] [*51-40-1,* anhydride]

Norepinephrine Tartrate Hydrate, when dried, contains not less than 97.0 % and not more than 102.0 % of *dl*-norepinephrine tartrate ($C_8H_{11}NO_3 \cdot C_4H_6O_6$).

Description Norepinephrine Tartrate Hydrate appears as white to pale brown or slightly reddish brown, crystalline powder.

Norepinephrine Tartrate Hydrate is freely soluble in acetic acid (100), very slightly soluble in water and practically insoluble in ethanol (95) or in ether.

Norepinephrine Tartrate Hydrate dissolves in dilute hydrochloric acid or in dilute acetic acid.

Norepinephrine Tartrate Hydrate gradually changes in color by air or light to brown.

Identification (1) Determine the infrared spectrum of Norepinephrine Tartrate Hydrate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Dissolve 50 mg of Norepinephrine Tartrate Hydrate in 10 mL of water and add one drop of iron (II) chloride: a strong green color is observed.

(3) To 1 mL of the solution from (2), add water to make 500 mL. Take 1 mL of this solution and make 1000 mL. To 10 mL of this solution, add 1.0 mL of 0.10 mol/L iodine solution, allow to stand for 5 minutes and add 2.0 mL of 0.10 mol/L sodium thiosulfate: the solution is colorless or pale pink.

Specific Optical Rotation $[\alpha]_D^{20}$: -10 ~ + -12° (0.50 g calculated on the dried basis, water, 100 mL, 100 mm).

Purity ***Arterenone***—Dissolve 0.2 g of Norepinephrine Tartrate Hydrate in water to make exactly 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: the absorbance at 310 nm is not more than 0.2.

Water 4.5 ~ 5.8 % (0.5 g, volumetric titration, direct titration)

Residue on Ignition Not more than 0.1 % (0.2 g)

Assay Weigh accurately about 0.25 g of Norepinephrine Tartrate Hydrate, previously dried, dissolve in 100 mL of acetic acid for nonaqueous titration by warming, if necessary, and titrate with 0.1 mL perchloric acid (indicator: 2 drops of methylrosaniline chloride TS) until the color changes from blue-purple through blue to blue-green. Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid
= 31.93 mg of $C_8H_{11}NO_3 \cdot C_4H_6O_6$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and at a room temperature.

Norepinephrine Tartrate Injection

Noradrenaline Tartrate Injection
Norepirenamine Tartrate Injection

Norepinephrine Tartrate Injection is an aqueous solution for injection. Norepinephrine Tartrate Injection contains not less than 90.0 % and not more than 115.0 % of the labeled amount of *dl*-norepinephrine ($C_8H_{11}NO_3$: 169.18).

Method of Preparation Prepare as directed under Injections, with Norepinephrine Tartrate Hydrate.

Description Norepinephrine Tartrate Injection is a clear and colorless liquid.

pH—3.0 ~ 4.5.

Identification (1) Perform the test as directed in Identification (2) under Norepinephrine Tartrate Hydrate.

(2) Pipet a volume of Norepinephrine Tartrate Injection, equivalent to 2 mg of norepinephrine according to the labeled amount, and add water to make 10 mL. Add 2.0 mL of 0.10 mol/L iodine solution, allow to stand for 5 minutes and add 3.0 mL of 0.10 mol/L sodium thiosulfate: the solution is colorless or pale pink.

Purity (1) ***Clarity and color of solution***—Pipet a suitable amount of Norepinephrine Tartrate Injection, transfer to a test tube and examine against a white background: no pink color or precipitate is observed. If a yellow color is observed, use this solution as the test solution. Add water to 2.0 mL of 0.1 mol/L iodine solution to make 500 mL and use this solution as the standard solution. Determine the absorption spectra of the standard solution and the test solution at 460 nm as directed under Ultraviolet-visible Spectrophotometry: the absorbance of the test solution is not greater than that of the standard solution.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 83.4 EU/mg of norepinephrine.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay Pipet a volume of Norepinephrine Tartrate Injection, equivalent to about 5 mg of *dl*-norepinephrine ($C_8H_{11}NO_3$), add dilute acetic acid (1 in 25) to make exactly 25 mL and use this solution as the test solution. Separately, weigh accurately a suitable amount of Norepinephrine Tartrate RS, dissolve in dilute acetic acid (1 in 25) to render the concentration of 0.4 mg/mL and use this solution as the test solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography under the following operating conditions and determine the peak areas of norepinephrine, A_T and A_S.

Amount (mg) of *dl*-norepinephrine ($C_8H_{11}NO_3$) in each mL of Norepinephrine Tartrate Injection

$$= \frac{\text{Concentration (mg/mL) of the standard solution}}{\text{Amount (mL) of Norepinephrine Tartrate Injection}} \times \frac{A_T}{A_S} \times 25 \times \frac{169.18}{337.28}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm)

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Dissolve 1.1 g of sodium 1-heptanesulfonate in 800 mL of water, add 200 mL of methanol, adjust the pH to 3.0 ± 0.1 with 1 mol/L phosphoric acid and filter through a membrane filter.

Flow rate: 2.0 mL/minute

System suitability

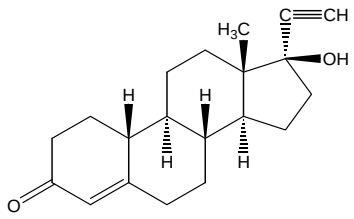
System performance: Weigh accurately a suitable portion each of isoproterenol and Norepinephrine Tartrate RS, dissolve in dilute acetic acid (1 in 25) to render the concentration of 0.4 mg/mL. When the procedure is run with 20 μL of this solution under the above operating conditions, the resolution between the peaks of norepinephrine and isoproterenol is not less than 4.0.

System repeatability: When the test is repeated 5 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of norepinephrine is not more than 2.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Storage—Light-resistant.

Norethisterone



$C_{20}H_{26}O_2$: 298.42

(8*R*,9*S*,10*R*,13*S*,14*S*,17*R*)-17-Ethynyl-17-hydroxy-13-methyl-1,2,6,7,8,9,10,11,12,14,15,16-dodecahydrocyclopenta[a]phenanthren-3-one [*68-22-4*]

Norethisterone, when dried, contains not less than 97.0 % and not more than 103.0 % of norethisterone ($C_{20}H_{26}O_2$).

Description Norethisterone is a white to pale yellow, crystalline powder and is odorless.
Norethisterone is sparingly soluble in ethanol (95), in acetone or in tetrahydrofuran, slightly soluble in ether and very slightly soluble in water.
Norethisterone is affected by light.

Identification (1) Take 2 mg of Norethisterone and add 2 mL of sulfuric acid: the solution shows a red-brown color and a yellow-green fluorescence. Add 10 mL of water to this solution cautiously: a yellow color is observed and a pale brown precipitate is produced.

(2) Take 25 mg of Norethisterone and add 3.5 mL of a solution of 50 mg of hydroxylamine hydrochloride and 50 mg of anhydrous sodium acetate in 25 mL of methanol. Heat under a reflux condenser in a water-bath for 5 hours, cool and add 15 mL of water. Filter the precipitate formed, wash with 1 mL to 2 mL of water, recrystallize from methanol and dry in a desiccator (in vacuum, silica gel) for 5 hours: the crystals melt between 112 °C and 118 °C.

Specific Optical Rotation $[\alpha]_D^{20}$: -32 ~ -37° (after drying, 0.25 g, acetone, 25 mL, 200 mm).

Melting Point 203 ~ 209 °C.

Purity ***Related substances***—Take 0.1 g each of Norethisterone and Norethisterone RS and prepare the test solution and the standard solution as directed in the Purity (2) Related substances i) under Norethisterone Acetate. Perform the test with these solutions as directed under the Thin-layer Chromatography. Develop the plate with a mixture of chloroform and methanol (95 : 5) to 3/4 of the total length and air-dry the plate. Spray evenly a mixture of methanol and sulfuric acid (7 : 3) and heat at 100 °C for 5 minutes: the principal spot obtained from the test solution and the principal spot obtained from the standard solution (1) are the same in R_f value, spots other than the principal spot obtained from the test solution are not more intense than the spots obtained from the standard solution (2) (0.5 %), and the total intensity of spots other than the principal spot is not more intense than the spot obtained from the standard solution (1) (1.5 %).

Loss on Drying Not more than 0.5 % (0.5 g, in vacuum, silica gel, 4 hours).

Residue on Ignition Not more than 0.1 % (0.5 g).

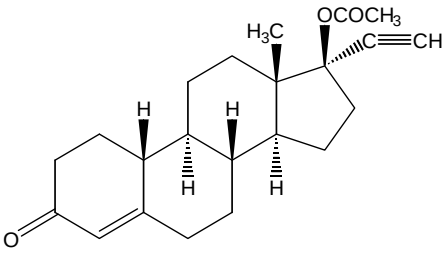
Assay Weigh accurately about 0.2 g of Norethisterone, previously dried, dissolve in 40 mL of tetrahydrofuran and add 10 mL of a solution of silver nitrate (1 in 20) and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 29.843 mg of $C_{20}H_{26}O_2$

Containers and Storage ***Containers***—Tight containers

Storage—Light-resistant.

Norethisterone Acetate



Norethindrone Acetate
Norethisterone Acetate $C_{22}H_{28}O_3$: 340.46

[(8*R*,9*S*,10*R*,13*S*,14*S*,17*R*)-17-Ethynyl-13-methyl-3-oxo-1,2,6,7,8,9,10,11,12,14,15,16-dodecahydrocyclopenta[a]phenanthren-17-yl] acetate [*51-98-9*]

Norethisterone Acetate contains not less than 97.0 %

and not more than 103.0 % of norethisterone acetate ($C_{22}H_{28}O_3$), calculated on the dried basis.

Description Norethisterone Acetate is a white to milky white crystalline powder and is odorless.
Norethisterone Acetate is soluble in ether or in ethanol (95) and practically insoluble in water.

Identification Determine the infrared spectra of Norethisterone Acetate and Norethisterone Acetate RS, both previously dried, as directed under the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: -30 ~ -35° (calculated on the dried basis, 0.50 g, ethanol (95), 25 mL, 100 mm)

Purity (1) ***Clarity of solution***—When 0.5 g of Norethisterone Acetate is dissolved in 25 mL of 1,4-dioxane, the solution is clear.

(2) ***Related substances***— (i) Dissolve 0.1 g of Norethisterone Acetate in chloroform to make exactly 10 mL and use this solution as the test solution. Separately, dissolve 0.1 of Norethisterone Acetate RS in chloroform to make exactly 10 mL and dilute this solution by the addition of chloroform to render the standard solutions (1), (2), (3) and (4) having the concentration of 150, 50, 30 and 10 μg in 1 mL, respectively. Perform the test with the standard and the test solution as directed under the Thin-layer Chromatography. Then, develop the plate with a mixture containing toluene and ethylacetate (1 : 1) to a distance of about 15 cm and air-dry the plate. Spray evenly with a mixture of methanol and sulfuric acid and heat the plate at 100 °C for 5 minutes. The spots other than the principal spot from the test solution are not more intense than the spot obtained from the standard solution (2). And the total intensity of the spots other than the principal spot from the test solution is not more intense than that from the standard solution (1).

(ii) Dissolve 62.5 mg of Norethisterone Acetate in the mobile phase to make exactly 25 mL and use this solution as the test solution. Pipet 1.0 mL of test solution, add the mobile phase to make exactly 100 mL and use this as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography. Determine each peak area of these solutions: the area of each peak of the test solution, except the pricipal peak, is not more than 1/2 of the peak area of the principal peak of the standard solution (0.5 %). And the total area of peaks of the test solution is not more than the area of the principal peak of the standard solution (1.0 %). Exclude the peak which has not more than 0.025 times of the area of the principal peak of the standard solution in the calculation of the total area.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 to 10 μm in paricle diameter).

Mobile phase: A mixture of acetonitrile and water (6:4).

Flow rate: 1.0 mL/minute.

System suitability

System performance: Dissolve 8 mg each of desoxycorticosterone acetate RS and Norethisterone Acetate RS in the mobile phase to make exactly 100 mL. When the test is performed with 20 μL of the solution as directed under the above operating conditions, desoxycorticosterone acetate and norethisterone acetate are eluted in this order with the resolution between their peaks being not less than 3.5.

Time span of measurement: About twice as long as the retention time of norethisterone acetate.

(3) ***Ethinyl-group***—Weigh accurately about 0.2 g of Norethisterone Acetate and dissolve in 40 mL of tetrahydrofuran. Add 10 mL of silver nitrate solution (1 in 10) and tritrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

The content of ethinyl group is between 7.13 % and 7.17 %

Each mL of 0.1 mol/L sodium hydroroxide VS
= 2.503 mg of −C≡CH

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

Assay Weigh accurately about 0.1 g each of Norethisterone Acetate and Norethisterone Acetate RS and dissolve in ethanol (95) to make exactly 200 mL each. Pipet 5.0 mL each of the solution and add ethanol (95) to make exactly 250 mL each. Use the solution as the test solution and the standard solution, respectively. Measure the absorbances of the test solution (A_T) and the standard solution (A_S) at the wavelength of 240 nm as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of norethisterone acetate } (C_{22}H_{28}O_3) = \text{Amount (mg) of Norethisterone Acetate RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Norethisterone Tablets

Norethindrone Tablets

Norethisterone Tablets contains not less than 90.0 % and not more than 110.0 % of the labeled amount of norethisterone ($C_{20}H_{26}O_2$: 298.42).

Method of Preparation Prepare as directed under the Tablets, with Norethisterone.

Identification Weigh a portion of powdered Norethisterone Tablets, equivalent to 50 mg of Norethisterone according to the labeled amount, add 15 mL of hexane, shake occasionally for 15 minutes, centrifuge the mixture and discard the hexane layer. Extract the residues with 10 mL of hexane twice, centrifuge and discard the hexane layer. To the residue, add 25 mL of chloroform, shake for 1 to 2 minutes and filter. Evaporate the filtrate until it is concentrated to 3 mL, add a suitable amount of hexane to make crystals and evaporate to dryness. Determine the infrared spectra of this residue and Norethisterone RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Dissolution Test Take 1 tablet of Norethisterone Tablets and perform the test at 75 revolutions per minute according to Method 2, using 500 mL of 0.1 mol/L hydrochloric acid TS containing 0.09 % sodium lauryl sulfate, degassed, as the dissolution solution. After 30 minutes from the start of the test, take not less than 20 mL of the dissolved solution and filter through a membrane filter with pore size of 0.45 μm. Discard the first 10 mL of the filtrate and use the subsequent filtrate as the test solution. Separately, weigh accurately about 35 mg of Norethisterone RS and place in a volumetric flask. Add 100 mL of methanol, sonicate to dissolve completely, cool to room temperature and add methanol to make exactly 500 mL. Pipet 2.0 mL of this solution, add the dissolution solution to make exactly 200 mL and use this solution as the standard solution. Perform the test with 100 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the peak area of norethisterone in each solution, A_T and A_S: the dissolution rate of Norethisterone Tablets in 30 minutes is not less than 80 % (Q).

Dissolution rate (%) with respect to the labeled amount of norethisterone ($C_{20}H_{26}O_2$)

$$= \text{Amount (mg) of Norethisterone RS} \times \frac{A_T}{A_S} \times \frac{1}{C}$$

C: Labeled amount (mg) of norethisterone ($C_{20}H_{26}O_2$) in 1 tablet

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of water and acetonitrile (3:2)

Flow rate: 1.5 mL/minute

System suitability

System repeatability: When the test is repeated 5 times with 100 μL each of the standard solution under the above operating conditions, the relative standard deviation of norethisterone is not more than 3.0 %.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately not less than 20 Norethisterone Tablets and powder. Weigh accurately a portion of the powder, equivalent to about 0.7 mg of norethisterone ($C_{20}H_{26}O_2$), add purified methanol, to make exactly 50 mL, shake occasionally and stand for 10 minutes. Filter the mixture, transfer 10.0 mL of the filtrate to a glass-stoppered flask, add 2.0 mL of isoniazid TS and stand for 30 minutes. Use this solution as the test solution. Separately, transfer 10.0 mL of the remaining filtrate to a suitable container, add 2.0 mL of methanol and use this solution as a control solution of the test solution. Pipet 10.0 mL of methanol to a glass-stoppered flask, add 2.0 mL of isoniazid TS, shake after capping and stand for 30 minutes. Use this solution as a control solution of the reagent solution. Separately, weigh accurately a portion of Norethisterone RS and dissolve in methanol to make a solution containing 14 μg per mL. Pipet 10.0 mL of this solution to a glass-stoppered flask, add 2.0 mL of isoniazid TS, shake after capping and stand for 30 minutes. Use this solution as the standard solution. Determine the absorbances, A_T, A_B and A_S, of the test solution, the control solution of the test solution and the standard solution, respectively, at 380 nm as directed under Ultraviolet-visible Spectrophotometry using methanol as a blank of the control solution of the test solution and using the control solution of the test solution as a blank of the reagent solution and the standard solution.

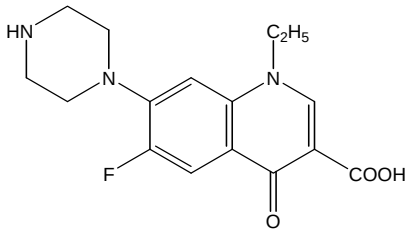
Amount (mg) of norethisterone ($C_{20}H_{26}O_2$)

$$= 0.05 \times C \times \frac{A_T - A_B}{A_S}$$

C: Concentration of norethisterone ($C_{20}H_{26}O_2$) in the standard solution (μg/mL).

Containers and Storage ***Containers***—Well-closed containers.

Norfloxacin



$C_{16}H_{18}FN_3O_3$: 319.33

1-Ethyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid [*70458-96-7*]

Norfloxacin, when dried, contains not less than 99.0 % and not more than 101.0 % of norfloxacin ($C_{16}H_{18}FN_3O_3$).

Description Norfloxacin is a white to pale yellow crystalline powder.
Norfloxacin is freely soluble in acetic acid (100), slightly soluble in ethanol (99.5) or in acetone, very slightly soluble in methanol, and practically insoluble in water.
Norfloxacin dissolves in dilute hydrochloric acid and in sodium hydroxide.
Norfloxacin is hygroscopic.
Norfloxacin is gradually colored by light.

Identification (1) Dissolve 10 mg of Norfloxacin and Norfloxacin RS in a solution of sodium hydroxide (1 in 250) to make 10 mL. To 5 mL each of these solutions add a solution of sodium hydroxide (1 in 250) to make 100 mL. Determine the absorption spectra of solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve a suitable amount of Norfloxacin and Norfloxacin RS in a suitable amount of acetone, evaporate the acetone under reduced pressure, and dry the residue. Determine the infrared spectra of the residues as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity (1) ***Sulfates***—Dissolve 1.0 g of Norfloxacin in 7 mL of 0.5 mol/L sodium hydroxide TS and 23 mL of water, and add 1 drop of phenolphthalein TS. Add gradually diluted hydrochloric acid (1 in 3) to this solution until the red color disappears, then add 0.5 mL of dilute hydrochloric acid, and cool in ice for 30 minutes. Filter through a glass filter (G4), and wash the residue with 10 mL of water. Combine the filtrate and the washing, and add 1 mL of dilute hydrochloric acid and water to make exactly 50 mL and perform the test. Prepare the control solution as follows. To 0.50 mL of 0.005 mol/L sulfuric acid VS add 7 mL of 0.5 mol/L sodium hydroxide TS and 1 drop of phenolphthalein TS, add diluted hydrochloric acid (1 in 3) until the red color disappears, then add 1.5 mL of dilute hydrochloric acid, 1 or 2 drops of bromophenol blue TS and water to make exactly 50 mL (not more than 0.024 %).

(2) ***Heavy metals***—Proceed with 2.0 g of Norfloxacin according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 15 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Norfloxacin according to Method 3, and perform the test (not more than 2 ppm).

(4) ***Related substances***—Perform the test without exposure to light, using light-resistant vessels. Dissolve 0.10 g of Norfloxacin in 50 mL of mixture of methanol and acetone (1 : 1), and use this solution as the test solution. Pipet 1.0 mL of the test solution, and add a mixture of methanol and acetone (1 : 1) to make exactly 100 mL. Pipet 2.0 mL of this solution, add a mixture of methanol and acetone (1 : 1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 20 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography (5 μm to 7 μm in particle diameter). Develop the plate with a mixture of methanol, chloroform, toluene, diethylamine and water (20 : 20 : 10 : 7 : 4) to a distance of about 9 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm and 366 nm): the number of the spot other than the principal spot from the test solution is not more than 2 and these spots are not more intense than the spot from the standard solution.

Loss on Drying Not more than 1.0 % (1 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

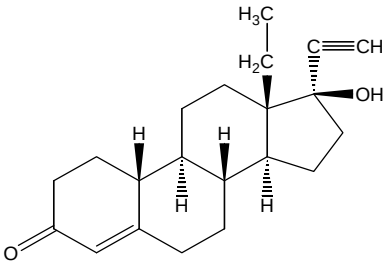
Assay Weigh accurately about 0.5 g of Norfloxacin, previously dried, dissolve in 50 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 31.933 mg of $C_{16}H_{18}FN_3O_3$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Norgestrel



$C_{21}H_{28}O_2$: 312.45

(8*R*,9*S*,10*R*,13*S*,14*S*,17*R*)-13-Ethyl-17-ethynyl-17-hydroxy-1,2,6,7,8,9,10,11,12,14,15,16-dodecahydrocyclopenta[a]phenanthren-3-one
[*6533-00-2*]

Norgestrel, when dried, contains not less than 98.0 % and not more than 101.0 % of norgestrel ($C_{21}H_{28}O_2$).

Description Norgestrel appears as white crystals or crystalline powder.
Norgestrel is soluble in chloroform or in tetrahydrofuran, sparingly soluble in ethanol (95), slightly soluble in ether and practically insoluble in water.

Identification (1) Dissolve 1 mg of Norgestrel in 2 mL of ethanol (95) and add 1 mL of sulfuric acid: a red-purple color is observed. With this solution, examine under ultraviolet light (main wavelength: 365 nm): the solution shows a orange fluorescence.

(2) Determine the infrared spectra of Norgestrel and Norgestrel RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 206 ~ 212 °C.

Purity (1) ***Heavy metals***—Take 1.0 g of Norgestrel, heat gently to carbonize, cool, add 10 mL of a solution of magnesium nitrate in ethanol (95) (1 in 10) and ignite the ethanol (95) to burn. After cooling, add 1 mL of sulfuric acid, proceed with this solution according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Dissolve 30 mg of Norgestrel in 5 mL of chloroform and use this solution as the test solution. Pipet 1.0 mL of the test solution, add chloroform to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane and ethyl acetate (2 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution is not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

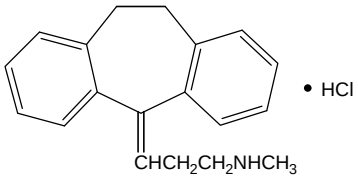
Residue on Ignition Not more than 0.2 % (0.5 g).

Assay Weigh accurately about 0.2 g of Norgestrel, previously dried, dissolve in 40 mL of tetrahydrofuran, add 10 mL of a solution of silver nitrate (1 in 20) and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 31.245 mg of $C_{21}H_{28}O_2$

Containers and Storage ***Containers***—Well-closed containers.

Nortriptyline Hydrochloride



$C_{19}H_{21}N \cdot HCl$: 299.84

3-(10,11-Dihydro-5*H*-dibenzo[a,d]cyclohepten-5-ylidene)-*N*-methylpropan-1-amine hydrochloride
[*894-71-3*]

Nortriptyline Hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of nortriptyline hydrochloride ($C_{19}H_{21}N \cdot HCl$).

Description Nortriptyline Hydrochloride is a white to yellow, crystalline powder, is odorless, or has a pale, characteristic odor.
Nortriptyline Hydrochloride is freely soluble in acetic acid (100) or in chloroform, soluble in ethanol (95), sparingly soluble in water and practically insoluble in ether.

pH—The pH of a solution of Nortriptyline Hydrochloride (1 in 100) is about 5.5.

Melting point—215 ~ 220 °C.

Identification (1) Take 5 mL of a solution of Nortriptyline Hydrochloride (1 in 100) and add 1 mL of bromine TS: the color of the test solution disappears.

(2) Take 5 mL of a solution of Nortriptyline Hydrochloride (1 in 100) and add 1 to 2 drops of a solution of quinhydrone in methanol (1 in 40): a red color gradually develops.

(3) Determine the absorption spectra of solutions of Nortriptyline Hydrochloride and Nortriptyline Hydrochloride RS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared spectra of Nortriptyline Hydrochloride and Nortriptyline Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(5) A solution of Nortriptyline Hydrochloride (1 in 100) responds to the Qualitative Tests for chloride.

Purity (1) ***Clarity and color of solution*—**Dissolve 0.10 g of Nortriptyline Hydrochloride in 10 mL of water: the solution is clear and colorless to very pale yellow.

(2) ***Heavy metals*—**Proceed with 1.0 g of Nortriptyline Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic*—**Prepare the test solution with 1.0 g of Nortriptyline Hydrochloride according to Method 3 and perform the test (not more than 2 ppm).

(4) ***Related substances*—**Dissolve 0.50 g of Nortriptyline Hydrochloride in 20 mL of chloroform and use this solution as the test solution. Pipet 2 mL of the test solution and add chloroform to make exactly 100 mL. Pipet 5 mL of this solution, add chloroform to make exactly 50 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 4 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, methanol and diethylamine (8 : 1 : 1) to a distance of about 15 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

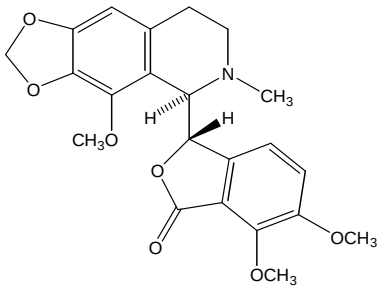
Assay Weigh accurately about 0.5 g of Nortriptyline Hydrochloride, previously dried, dissolve in 5 mL of acetic acid (100), add 50 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.984 mg of $C_{19}H_{21}N \cdot HCl$

Containers and Storage ***Containers*—**Well-closed containers.

***Storage*—**Light-resistant.

Noscapine



Narcotine $C_{22}H_{23}NO_7$: 413.4

(3*S*)-6,7-Dimethoxy-3-((5*R*)-4-methoxy-6-methyl-5,6,7,8-tetrahydro-[1,3]dioxolo[4,5-g]isoquinolin-5-yl)isobenzofuran-1(3*H*)-one [*128-62-1*]

Noscapine, when dried, contains not less than 98.5 % and not more than 101.0 % of noscapine ($C_{22}H_{23}NO_7$).

Description Noscapine appears as white crystals or crystalline powder and is odorless and tasteless.
Noscapine is very soluble in acetic acid (100), slightly soluble in ethanol (95) or in ether and practically insoluble in water.

Identification (1) Determine the absorption spectra of solutions of Noscapine and Noscapine RS (1 in 20000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Noscapine and Noscarpine RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +42 ~ +48° (after drying, 0.5 g, 0.1 mol/L hydrochloric acid TS, 25 mL, 100 nm).

Melting Point 174 ~ 177 °C.

Purity (1) ***Chloride*—**Dissolve 0.7 g of Noscapine in 20 mL of acetone, add 6 mL of dilute nitric acid and water to make 50 mL and perform the test with this solution. Prepare the control solution as follows: to 0.4

mL of 0.01 mol/L hydrochloric acid VS, add 20 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.02 %).

(2) ***Heavy metals***—Proceed with 2.0 g of Noscapine according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Morphine***—Dissolve 10 mg of Noscapine in 1 mL of water and 5 mL of 1-nitroso-2-naphthol TS with shaking, add 2 mL of a solution of potassium nitrate (1 in 10) and warm at 40 °C for 2 minutes. Then add 1mL of a solution of sodium nitrate (1 in 5000) and warm at 40 °C for 5 minutes. After cooling, shake the solution with 10 mL of chloroform, centrifuge and collect the aqueous layer: the solution so obtained has no more color than a pale red.

(4) ***Related substances***—Dissolve 0.7 g of Noscapine in 50 mL of acetone and use this solution as the test solution. Pipet 5.0 mL of the test solution and add acetone to make exactly 50 mL. Pipet 5.0 mL of this solution, add acetone to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, toluene, ethanol (99.5) and ammonia solution (28) (60 : 60 : 9 : 2) to a distance of about 10 cm and air-dry the plate. Spray evenly dilute bismuth subnitrate-potassium iodide TS for spray on the plate: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (2 g, 105 °C, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

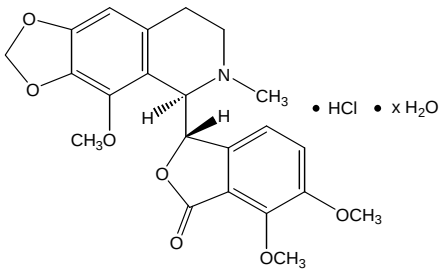
Assay Weigh accurately about 0.8 g of Noscapine, previously dried, dissolve in 30 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (indicator: 3 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 41.34 mg of $C_{22}H_{23}NO_7$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Noscapine Hydrochloride Hydrate



Narcotine Hydrochloride $C_{22}H_{23}NO_7 \cdot HCl \cdot xH_2O$

(3*S*)-6,7-Dimethoxy-3-((5*R*)-4-methoxy-6-methyl-5,6,7,8-tetrahydro-[1,3]dioxolo[4,5-g]isoquinolin-5-yl)isobenzofuran-1(3*H*)-one hydrate hydrochloride [*912-60-7*]

Noscapine Hydrochloride Hydrate, when dried, contains not less than 98.0 % and not more than 101.0 % of noscapine hydrochloride ($C_{22}H_{23}NO_7 \cdot HCl$: 449.88).

Description Noscapine Hydrochloride Hydrate appears as colorless or white crystals or crystalline powder, is odorless and has a bitter taste.
Noscapine Hydrochloride Hydrate is freely soluble in water, in acetic acid (100) or in acetic anhydride, soluble in ethanol (95) and practically insoluble in ether.

Identification (1) Take 1 mg of Noscapine Hydrochloride Hydrate and add 1 drop of formaldehyde-sulfuric acid TS: a purple color, changing to yellow-brown, is produced.

(2) Take 1 mg of Noscapine Hydrochloride Hydrate and add 1 drop of a solution of ammonium vanadate in sulfuric acid (1 in 200): an orange color is produced.

(3) Dissolve 20 mg of Noscapine Hydrochloride Hydrate in 1 mL of water and add 3 drops of sodium acetate TS: a white, flocculent precipitate is produced.

(4) Dissolve 1 mg of Noscapine Hydrochloride Hydrate in 1 mL of diluted sulfuric acid (1 in 35), shake with 5 drops of a solution of chromotropic acid (1 in 50) and add 2 mL of sulfuric acid drop-wise: a purple color is produced.

(5) Dissolve 0.1 g of Noscapine Hydrochloride Hydrate in 10 mL of water, make the solution alkaline with ammonia TS and shake with 10 mL of chloroform. Separate the chloroform layer, wash with 5 mL of water and filter. Evaporate most of the filtrate in a water-bath, add 1 mL of purified methanol and evaporate to dryness. Dry the residue at 105 °C for 4 hours: the residue so obtained melts between 174 °C and 177 °C.

(6) Make a solution of Noscapine Hydrochloride Hydrate (1 in 50) alkaline with ammonia TS and filter the precipitate. Acidify the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests (2) for chloride.

Purity ***Morphine***—Dissolve 10 mg of Noscapine Hydrochloride Hydrate in 1 mL of water, add 5mL of 1-nitroso-2-naphthol TS and 2 mL of a solution of potassium nitrate (1 in 10) and warm at 40 °C for 2 minutes. Add 1 mL of a solution of sodium nitrite (1 in 5000) and warm at 40 °C for 5 minutes. After cooling, shake the mixture with 10 mL of chloroform, centrifuge and separate the aqueous layer: the solution so obtained is not more colored than a pale red color.

Loss on Drying Not more than 9.0 % (0.5 g, 120 °C, 4 hours).

Residue on Ignition Not more than 0.5 % (1 g).

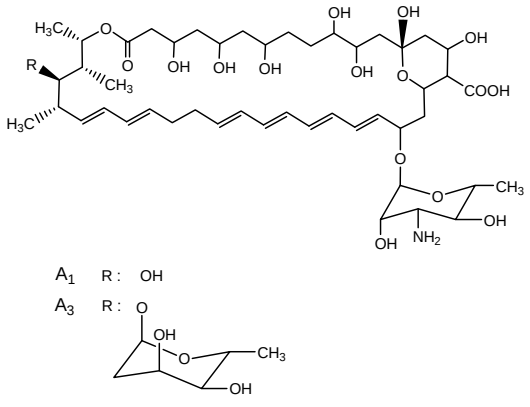
Assay Weigh accurately about 0.5 g of Noscapine Hydrochloride Hydrate, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 44.99 mg of $C_{22}H_{23}NO_7 \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Nystatin



Nystatin A_1 $C_{47}H_{75}NO_{17}$: 926.10

(4*E*,6*E*,8*E*,10*E*,14*E*,16*E*,18*S*,19*R*,20*R*,21*S*,35*S*)-3-[(2*S*,3*S*,4*S*,5*S*,6*R*)-4-Amino-3,5-dihydroxy-6-methyloxan-2-yl]oxy-19,25,27,29,32,33,35,37-octahydroxy-18,20,21-trimethyl-23-oxo-22,39-dioxabicyclo[33.3.1]nonatriaconta-4,6,8,10,14,16-hexaene-38-carboxylic acid [*1400-61-9*]

Nystatin is a mixture of polyene macrolide substances having antifungal activity produced by the growth of *Streptomyces noursei*.

Nystatin contains not less than 4600 units (potency) per mg of nystatin A_1 ($C_{47}H_{75}NO_{17}$), calculated on the dried basis. One unit is equivalent to 0.27 μg of nystatin.

Description Nystatin is a white to pale brown powder.

Nystatin is soluble in formamide, sparingly soluble in methanol, slightly soluble in ethanol (95) and very slightly soluble in water.

Nystatin dissolves in sodium hydroxide TS.

Identification (1) Weigh 1 mg of Nystatin, dissolve in 5 mL of water and 1 mL of sodium hydroxide TS, heat for 2 minutes and allow to cool. To this solution, add 3 mL of a solution of 4-aminoacetophenone in methanol (1 in 200) and 1 mL of hydrochloric acid: a red-purple color is observed.

(2) To 10 mg of each Nystatin and Nystatin RS, add 0.25 mL of sodium hydroxide TS and 50 mL of diluted methanol (4 in 5), heat at not exceeding 50 °C to dissolve, then add diluted methanol (4 in 5) to make 500 mL. Determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry, both spectra exhibit similar intensities of absorption at the same wavelengths.

pH The pH of a suspension obtained by suspending 0.3 g of Nystatin in 10 mL of water is between 6.5 and 8.0.

Purity ***Heavy metals***—Proceed with 1.0 g of Nystatin according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

Abnormal Toxicity It meets the requirement when Nystatin is used in preparations other than those for skin application. Suspend and dissolve a portion of Nystatin, equivalent to 600 IU, in 5 % Arabic gum solution and inject into the peritoneal cavity of each of 5 healthy mice weighing 17 g to 24 g. Use animals in which no abnormalities are observed for not less than 5 days prior to the test. No animals die during the 24 hour post-dosage observation. If 1 animal dies, repeat the test with 5 animals: no animals die during the 24 hour observation.

Loss on Drying Not more than 5.0 % (0.3 g, in vacuum, 60 °C, 3 hours).

Content Ratio of Nystatins Weigh accurately about 20 mg each of Nystatin and Nystatin RS, dissolve in dimethyl sulfoxide to make exactly 50 mL and use these solutions as the test solution and the standard solution. Keep the test solution and the standard solution under refrigeration with protection from light and use within 24 hours. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine each peak area.

Determine the content by the area percentage method: the content of nystatin A_1 is not less than 85.0 % and the content of other substances is not more than 4.0 %. Exclude peaks with a retention time of less than 2 minutes.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 304 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 150 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30 °C

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: A mixture of 0.05 mol/L ammonium acetate solution and acetonitrile (71 : 29)

Mobile phase B: A mixture of acetonitrile and 0.05 mol/L ammonium acetate solution (60 : 40)

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-25	100	0
25-35	100→0	0→100
35-40	0	100
40-45	0→100	100→0
45-50	100	0

Flow rate: 1 mL/minute

System suitability

System performance: Weigh accurately about 20 mg of Nystatin RS, dissolve in 25 mL of methanol and add water to make 50 mL. To 10 mL of this solution, add 2 mL of dilute hydrochloric acid and allow to stand for 1 hour at room temperature. When the procedure is run with 20 μL of this solution under the above operating conditions, the resolution between the two main peaks is not less than 3.5 and the retention time of nystatin A_1 is about 14 minutes.

Assay ***The Cylinder-plate method*** (1) Agar media for seed and base layer- Use the culture medium in I 2 1) (4) ① under Microbial Assay for Antibiotics.

(2) Test organism- *Saccharomyces cerevisiae* ATCC 9763

(3) Weigh accurately a portion of Nystatin, equivalent to about 60000 units, dissolve in *N,N*-dimethylformamide to make a solution containing 3000 units per mL and use this solution as the test stock solution. Pipet a suitable amount of this solution, dilute with 1 % phosphate buffer (pH 6.0) to make solutions containing 300 units and 150 units per mL, and use these solutions as the high concentration test solution and the low concentration test solution, respectively. Use light-resistant vessels. Separately, weigh accurately a portion of Nystatin RS (previously dried in vacuum at not more than 0.67 kPa and 40 °C for 2 hours), equivalent to about 60000 units, and dissolve in *N,N*-dimethylformamide to make a standard stock solution containing 300 units per mL. Keep the standard stock solution at not exceeding 5 °C and use within 3 days. Pipet a suitable volume of this standard stock solution, dilute with 1 % phosphate buffer (pH 6.0) to make solutions containing 300 units and 150 units per mL, and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively. Perform the test with these solutions according to the Cylinder-plate method (I 8) as directed under Microbial Assay for Antibiotics.

Containers and Storage ***Containers*****—**Tight containers.

Storage**—**Light-resistant, and in a cold place.

Nystatin Suppositories

Nystatin Suppositories are vaginal suppository capsules. Nystatin Suppositories contain not less than 90.0 % and not more than 120.0 % of the labeled amount of nystatin ($C_{47}H_{75}NO_{17}$: 926.10).

Method of Preparation Prepare into capsules as directed under Suppositories, with Nystatin.

Identification (1) Transfer 2 g of Nystatin Suppositories to a centrifuge tube, add 20 mL of ether, shake vigorously and centrifuge. Discard the clear supernatant liquid, add 10 mL of ether and centrifuge in the same manner. Discard the clear supernatant liquid, dissolve the residue in 25 mL of 5 % acetic acid-methanol solution and use this solution as the test stock solution. Take 1 mL of the test stock solution and add methanol to make 200 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima at 292 nm, 305 nm and 319 nm.

(2) Use the test stock solution from Identification (1) as the test solution. Separately, weigh about 25 mg of Nystatin RS, dissolve in 5 % acetic acid-methanol solution to make 25 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 25 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (31) (4:2:1) and air-dry the plate. Examine the spots (R_f value: about 0.45) of the test solution and the standard solution under ultraviolet light (main wavelength: 254 nm). Examine the spots again under ultraviolet light (main wavelength: 350 nm): a blue fluorescence is observed.

pH Dissolve a portion of Nystatin Suppositories, equivalent to 200000 units (potency), in 100 mL of

water by warming: the pH of the solution is between 5.5 and 6.5.

Disintegration Test It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay ***The Cylinder-plate method*** Perform the test as directed in the Assay under Nystatin. Weigh accurately a portion of Nystatin Suppositories, equivalent to about 250000 units (potency) according to the labeled potency, add 60 mL of petroleum ether, shake vigorously and centrifuge. Discard the clear supernatant liquid and dissolve the residue in *N,N*-dimethylformamide to make a solution containing 10000 units (potency) per mL. Pipet a suitable amount of this solution, dilute with 10 % phosphate buffer (pH 6.0) to make solutions containing 100 units (potency) and 50 units (potency) per mL, and use these solutions as the high concentration test solution and the low concentration test solution, respectively. Separately, weigh accurately a portion of Nystatin RS, equivalent to about 250000 units (potency) and dissolve in *N,N*-dimethylformamide to make a standard stock solution containing 10000 units (potency) per mL. Pipet a suitable amount of the standard stock solution, dilute with 10 % phosphate buffer (pH 6.0) to make solutions containing 100 units (potency) and 50 units (potency) per mL, and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

Containers and Storage ***Containers*****—**Tight containers.

Storage**—**Light-resistant.

Nystatin Syrup

Nystatin Syrup contains not less than 90.0 % and not more than 120.0 % of the labeled amount of nystatin ($C_{47}H_{75}NO_{17}$: 926.10).

Method of Preparation Prepare as directed under Syrups, with Nystatin.

Identification Take 2 mL of Nystatin Syrup, previously mixed well by shaking, dilute with 2 mL of water, add 2 drops of phosphomolybdotungstic acid TS and allow to stand for 1 hour: a green color is observed.

pH 4.5 ~ 6.0. When Nystatin Syrup contains glycerin, the pH is between 6.0 and 7.5.

Uniformity of Dosage Units (divided) It meets the requirement.

Assay Perform the test as directed in the Assay under Nytstatin. Pipet a suitable volume of Nystatin Syrup, transfer to a blender containing a sufficient amount of *N,N*-dimethylformamide and blend on high speed for 3 to 5 minutes to form a homogeneous liquid. Add *N,N*-dimethylformamide to make a solution containing 3000 units (potency) per mL. Pipet a suitable volume of this solution and dilute with 1 % phosphate buffer (pH 6.0) to make the concentration of (3) and use this solution as the test solution.

Containers and Storage ***Containers*****—**Tight containers.

Storage**—**Light-resistant.

Nystatin Tablets

Nystatin Tablets contain not less than 90.0 % and not more than 120.0 % of the labeled amount of nystatin ($C_{47}H_{75}NO_{17}$: 926.10).

Method of Preparation Prepare as directed under Tablets, with Nystatin.

Identification Weigh a portion of powdered Nystatin Tablets, equivalent to 10 mg of nystatin, add 5 mL of water and shake well. Add 2 drops of phosphomolybdotungstic acid TS and allow to stand for 1 hour: a green color is observed.

Loss on Drying Not more than 5.0 % (0.1 g, 0.7 kPa, 60 °C, 3 hours)

Disintegration Test It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Perform the test as directed in the Assay under Nystatin. Weigh accurately and powder not less than 20 Nystatin Tablets. Weigh a portion of the powder, equivalent to about 600000 units (potency) according to the labeled amount, transfer to a blender, add 150 mL of *N,N*-dimethylformamide and blend for 2 minutes. Add *N,N*-dimethylformamide to make exactly 200 mL and filter or centrifuge, if necessary. Pipet a suitable volume of this solution, dilute with 1 % phosphate buffer (pH 6.0) to make the concentration of (3) and use this solution as the test solution.

Containers and Storage ***Containers*****—**Tight containers.

Storage**—**Light-resistant.

Nystatin Vaginal Tablets

Nystatin Vaginal Tablets contain not less than 90.0 % and not more than 120.0 % of the labeled amount of nystatin ($C_{47}H_{75}NO_{17}$: 926.10).

Method of Preparation Prepare as directed under Tablets, with Nystatin.

Identification Weigh a portion of powdered Nystatin Vaginal Tablets, equivalent to 10 mg of nystatin, add 5 mL of water and shake well. Add 2 drops of phosphomolybdotungstic acid TS and allow to stand for 1 hour: a green color is observed.

Loss on Drying Not more than 5.0 % (0.1 g (finely powdered), 0.7 kPa, 60 °C, 3 hours).

Disintegration Test It meets the requirement when the test is performed as directed in Suppositories under Disintegration.

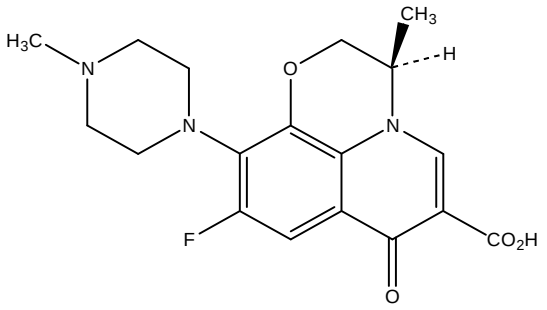
Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Nystatin Tablets.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Ofloxacin



and enantiomer

$C_{18}H_{20}FN_3O$: 361.37

7-Fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-azatricyclo[7.3.1.0^{5,13}]trideca-5(13),6,8,11-tetraene-11-carboxylic acid [*82419-36-1*]

Ofloxacin, when dried, contains not less than 99.0 % and mot more than 101.0 % of ofloxacin ($C_{18}H_{20}FN_3O$).

Description Ofloxacin appears as pale yellowish white to light yellowish white, crystals or crystalline powder.

Ofloxacin is freely soluble in acetic acid (100), slightly soluble in water, and very slightly soluble in acetonitrile and in ethanol (99.5).

A solution of Ofloxacin in sodium hydroxide TS (1 in 20) does not show optical rotation.

Ofloxacin is changed in color by light.

Melting point—About 265 °C (with decomposition).

Identification (1) Determine the absorption spectra of the solutions of Ofloxacin and Ofloxacin RS in 0.1 mol/L hydrochloric acid TS (1 in 150000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Ofloxacin and Ofloxacin RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +74.5 ~ +78.0° (1 g, water, 100 mL, 100 mm).

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Ofloxacin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) ***Arsenic***—Proceed with 2.0 g of Ofloxacin according to Method 2, and perform the test (not more than 1 ppm).

(3) ***Related substances***—Perform this procedure without exposure to light. Dissolve 10 mg of Ofloxacin in 50 mL of a mixture of water and acetonitrile (6 : 1), and use this solution as the test solution. Pipet 1 mL of the test solution, and add a mixture of water and acetonitrile (6 : 1) to make exactly 20 mL. Pipet 1 mL of this solution, add a mixture of water and acetonitrile (6 : 1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than ofloxacin from the test solution is not more than 0.4 times the peak are of ofloxacin from the standard solution, and the total area of the peaks other than ofloxacin from the test solution is not more than the peak area from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 294 nm).

Column: A stainless steel column 4.6 mm in internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45 °C.

Mobile phase: Dissolve 7.0 g of sodium perchlorate monohydrate and 4.0 g of ammonium acetate in 1300 mL of water, adjust the pH to 2.2 with phosphoric acid, and add 240 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention

time of ofloxacin is about 20 minutes.

System suitability

Test for required detectability: Measure 1 mL of the standard solution, and add a mixture of water and acetonitrile (6 : 1) to make exactly 20 mL. Confirm that the peak area of ofloxacin obtained from 10 μL of this solution is equivalent to 4 to 6 % of that from 10 μL of the standard solution.

System performance: To 0.5 mL of the test solution, add 1 mL of a solution of ofloxacin demethyl substance in a mixture of water and acetonitrile (6 : 1) (1 in 20000) and a mixture of water and acetonitrile (6 : 1) to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ofloxacin demethyl substance and ofloxacin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ofloxacin is not more than 2.0 %.

Time span of measurement: About 1.8 times as long as the retention time of ofloxacin beginning after the solvent peak.

Loss on Drying Not less than 0.2 % (1 g, 105 °C, 4 hours)

Residue on Ignition Not more than 0.1 % (1 g).

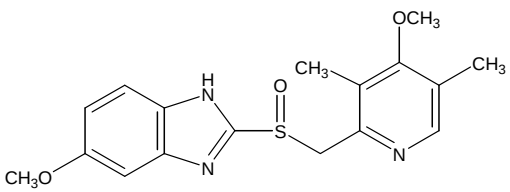
Assay Weigh accurately about 0.3 g of Ofloxacin, previously dried, dissolve in 100 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 36.14 mg of $C_{18}H_{20}FN_3O_4$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Omeprazole



$C_{17}H_{19}N_3O_3S$: 345.42

5-Methoxy-2-((4-methoxy-3,5-dimethylpyridin-2-yl) methylsulfinyl)-1*H*-benzo[d]imidazole [*73590-58-6*]

Omeprazole contains not less than not less than 98.0 % and not more than 102.0 % of omeprazole ($C_{17}H_{19}N_3O_3S$), calculated on the dried basis.

Description Omeprazole appears as white to pale red-purple powder.

Omeprazole is soluble in dichloromethane, in methanol or in ethanol (95) and practically insoluble in water.

Melting point—About 150 °C (with decomposition).

Identification (1) To 1 mL each of solutions of Omeprazole and Omeprazole RS in ethanol (99.5) (1 in 1000) add pH 7.4 phosphate buffer solution to make 50 mL, and determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Omeprazole and Omeprazole RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Perform the test with Omeprazole according to the Related substances (i) under the Purity: the spots obtained from the test solution and the standard solution have the same R_f value.

Purity (1) ***Clarity of solution***—Dissolve 0.2 g of Omeprazole in 10 mL of methanol: the solution is clear. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry, using dichloromethane as the blank: the absorbance at 440 nm is not more than 0.10.

(2) ***Heavy metals***—Proceed with 2.0 g of Omeprazole according to Method 2 under Heavy Metals Limit Test, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—(i) Weigh 1.0 g of Omeprazole and dissolve in a mixture of dichloromethane and methanol (1 : 1) to make exactly 20 mL. Pipet 1.0 mL of this solution, add a mixture of dichloromethane and methanol (1 : 1) to make exactly 200 mL and use this solution as the test solution. Separately, weigh accurately 15 mg of Omeprazole RS, dissolve in a mixture of dichloromethane and methanol (1 : 1) to make exactly 100 mL and use this solution as the standard solution (1). Pipet 10.0 mL of this solution, add a mixture of dichloromethane and methanol (1 : 1) to make exactly 30 mL and use this solution as the standard solution (2). Perform the test with the test solution and the standard solutions (1) and (2) as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solutions (1) and (2) on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, saturated with ammonia, dichloromethane and 2-propanol (2 : 2 : 1) to

a distance of about 15 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution (1) (0.3 %) and total intensity of the spots other than the principal spot of the test solution is not more than 1.0 % when compared to the principal spots from the standard solutions (1) and (2).

(ii) Weigh accurately 16 mg of Omeprazole, dissolve in the mobile phase to make exactly 100 mL and use this solution as the test solution (make this solution at the time of use). Determine the amount of the related substances with 40 μL each of the test solution and the mobile phase as directed under Liquid Chromatography according to the following operating conditions. The area of each peak which is not obsereved in the mobile phase other than the principal peak compared to the total area is not more than 0.3 % and the total area of the peaks which are not obsereved in the mobile phase is not more than 1.0 %.

Operating conditions

Detector, column, mobile phase and flow rate: Proceed as directed under the Assay.

Time span of measurement: Not less than twice of the retention time of omeprazole.

Loss on Drying Not more than 0.2 % (1.0 g, in vacuum, P_2O_5, 50 °C, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.1 g of Omeprazole and Omeprazole RS and dissolve in 50 mL each of a mixture of 0.01 mol/L of sodium borate and acetonitrile (3 : 1) to make exactly 50 mL. Pipet 5.0 mL each of these solutions, add 0.01 mol/L sodium borate and acetonitrile (3 : 1) to make exactly 50 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and calculate the ratio, A_T and A_S, of the peak area of Omeprazole for the test solution and the standard sollution, respectively.

$$\text{Amount (mg) of omeprazole } (C_{17}H_{19}N_3O_3S) = \text{Amount (mg) of Omeprazole RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of the phosphate buffer and acetonitrile (3 : 1).

Flow rate: 0.8 mL/minute.

System suitability

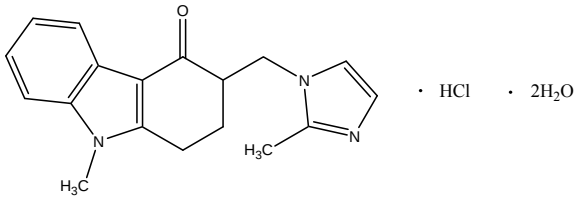
System repeatability: Take 10 mL of the standard solution, add 0.01 mol/L sodium borate and acetonitrile mixture (3 : 1) to make 20 mL. When the test is repeated 6 times with 20 μL of this solution according to the above operating conditions, the relative standard deviation of the peak areas of omeprazol is not more than 1.0 %.

Phosphate buffer—Dissolve 0.725 g of sodium dihydrogen phosphate dihydrate and 4.472 g of anhydrous disodium hydrogen phosphate in 300 mL of water and add water to make 1000 mL. Take 250 mL of this solution and add water to make 1000 mL.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and in a cold place.

Ondansetron Hydrochloride Hydrate



$C_{18}H_{19}N_3O \cdot HCl \cdot 2H_2O$: 365.85

9-Methyl-3-[(2-methyl-1*H*-imidazol-1-yl)methyl]-2,3,4,9-tetrahydro-1*H*-carbazol-4-one dihydrate hydrochloride

Ondansetron Hydrochloride Hydrate contains not less than 98.0 % and not more than 102.0 % of ondansetron hydrochloride ($C_{18}H_{19}N_3O \cdot HCl$: 329.82), calculated on the anhydrous basis.

Description Ondansetron Hydrochloride Hydrate appears as white powder. Ondansetron Hydrochloride Hydrate is soluble in water or methanol, sparingly soluble in ethanol (95), slightly soluble in 2-propanol or in dichloromethane, and very slightly soluble in chloroform or in ethyl acetate.

Identification (1) Determine the infrared spectra of Ondansetron Hydrochloride Hydrate and Ondansetron Hydrochloride Hydrate RS, previously dried, as directed in the paste method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Dissolve 20 mg of Ondansetron Hydrochloride Hydrate in 2 mL of water, mix with 1 mL of 2 mol/L nitric acid, and filter. The filtrate responds to the Qualitative Tests (2) for chlorides.

Purity (1) ***Related substance I***—Weigh accurately about 50 mg of Ondansetron Hydrochloride Hydrate, add mobile phase to make exactly 100 mL and use this solution as the test solution. Separately, weigh accurately about 4.0 mg of Ondansetron Related Substance I (1,2,3,9-Tetrahydro-9-methyl-3-methylene-*4H*-carbazol-4-one) RS, dissolve in mobile phase to make 100 mL, pipet 1.0 mL of this solution, add mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the test and the standard solution as directed under Liquid Chromatography according to the following conditions, determine each peak area, A_T and A_S respectively, and calculate the percentage of related substance I in the portion of ondansetron hydrochloride by the formula (not more than 0.1 %).

$$\text{Amount (\%) of ondansetron related substance I} = 10 \times \frac{C}{W} \times \frac{A_T}{A_S}$$

C: Concentration (μg/mL) of ondansetron related substance I in the standard solution.

W: Amount (mg) of Ondansetron Hydrochloride Hydrate.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 328 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 20 cm in length, packed with nitrile silica gel for Liquid Chromatography (3 to 10 μm in particle diameter).

Mobile phase: A mixture of phosphate buffer solution and acetonitrile (80 : 20).

Flow rate: 1.5 mL/minute

System suitability

System performance: Dissolve 6 mg of Ondansetron Related Substance I RS and 10 mg of Ondansetron Related Substance II (1,2,3,9-Tetrahydro-9-methyl-4*H*-carbazol-4-one) RS in mobile phase to make 100 mL. Pipet 1.0 mL of this solution and add mobile phase to make 100 mL. When the procedure is run with 20 μL of the this solution under the above operating condition, the relative retention times of ondansetron related substance I and ondansetron related substance II are about 1 and 0.8, respectively, with resolution between their peaks being not less than 1.5. When the procedure is run with 20 μL of the standard solution under the above operating condition, the number of theoretical plate is not less than 400.

System repeatability: When the test is repeated 5 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ondansetron is not more than 2.0 %.

Phosphate buffer solution—To 0.02 mol/L monobasic potassium phosphate buffer add 1 mol/L sodium hydroxide TS to adjust pH of 5.4.

(2) ***The other related substances***—(i) Weigh accurately 125 mg of Ondansetron Hydrochloride Hydrate, add methanol to make exactly 10 mL and use this solution as the test solution. Separately, weigh accurately 25 mg of Ondansetron Hydrochloride Hydrate RS, add methanol to make exactly 100 mL. Quantitatively dilute this solution with methanol to obtain those standard solutions, designated below by letter, having the following compositions:

Standard solutions	Dilution	Concentration (μg/mL)	Ratio (%) of the sample
1	1 in 5	50	0.4
2	1 in 10	25	0.2
3	1 in 20	12.5	0.1

Separately, dissolve 1.0 mg of Ondansetron Related Substance III {3-[(Dimethylamino)methyl]-1,2,3,9-tetra-hydro-9-methyl-*4H*-carbazol-4-one} RS in methanol to make exactly 10 mL and use this solution as the resolution solution. Also dissolve 1.0 mg of Ondansetron Related Substance IV {6,6'-Methylene-*bis*-[(1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-*1H*-imidazol-1-yl)-methyl]-*4H*-carbazol-4-one} RS in methanol to make exactly 10 mL and use this solution as the identification solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 20 μL each of the test solution and standard solutions and 10 μL of the identification solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. For system suitability test, spot 20 μL of the test solution and 10 μL of the identification solution on the same place of the plate. Develop the plate with a mixture of chloroform, ethyl acetate, methanol and ammonia solution (28) (90 : 50 : 40 : 1) to a distance of about 15 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm) and compare the spots other than the principal spot from the test solution with the principal spot from the standard solution: complete resolution of the three components of the resolution solution spot is found, and any spot other than the principal spot from the chromatogram of the test solution, having the same R_f value as that of the principal spot from the identification solution, is not larger or more intense than the principal spot from the standard solution 1 (0.4 %); no other secondary spot from the test solution is larger or more intense than the principal spot from the standard solution (2) (0.2 %). The total intensity of all spots other than the principal spot from the test solution is not more than 1.0 %.

(ii) Proceed as directed under Assay for the test solution and the standard solutions, and operating conditions. Perform the test with exactly 10 μL of the test solution as directed under Liquid Chromatography, and calculate the percentage of each peak area taken by the formula: the amount of each related substance is not

more than 0.2 % and the total amount of all the related substances is not more than 0.5 %.

$$\text{Amount (\%) of each related substance} = 100 \times \frac{A_i}{A_S}$$

A_i: Peak area for each related substance in the test solution
A_S: Total area of all peaks of each related substances

Water 9.0 ~ 10.5 % (0.5 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 45 mg of Ondansetron Hydrochloride Hydrate, dissolve in mobile phase to make exactly 50 mL, pipet 5.0 mL of this solution, add mobile phase to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately about 9.0 mg of Ondansetron Hydrochloride Hydrate RS (previously determine the content of water), dissolve in mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform the test with exactly 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of ondansetron, A_T and A_S, respectively.

$$\text{Amount (mg) of Ondansetron Hydrochloride} = 500 \times C \times \frac{A_T}{A_S}$$

C : Concentration (mg/mL) of ondansetron hydrochloride in the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 216 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 20 cm in length, packed with nitrile silica gel for Liquid Chromatography (3 to 10 μm in particle diameter).

Mobile phase: The mixture of pH 5.4 phosphate buffer solution and acetonitrile (50 : 50).

Flow rate: 1.5 minute/minute.

System suitability

System performance: Dissolve 9 mg of Ondansetron Hydrochloride RS and 5 mg of Ondansetron Related Substance III RS in mobile phase to make exactly 100 mL. When the procedure is run with 10 μL of this solution under the above operating condition, the relative retention times of ondansetron and ondansetron related substance III are 1.0 and 1.1, respectively; with the resolution, R, between their peaks is not less than 1.5. When the procedure is run with 10 μL of the standard solution, the symmetry factor of the peak of ondansetron is not more than 2.0.

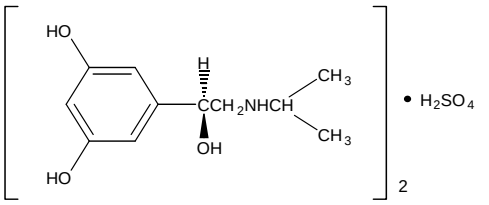
System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ondansetron is not more than 1.5 %.

Phosphate buffer solution—To 0.02 mol/L potassium dihydrogen phosphate buffer solution add 1 mol/L sodium hydroxide TS to adjust pH 5.4.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Orciprenaline Sulfate



and enantiomer

$(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4$: 520.59

bis{5-[1-Hydroxy-2-(propan-2-ylamino)ethyl]benzene-1,3-diol} sulfate [*5874-97-5*]

Orciprenaline Sulfate contains not less than 98.5 % and not more than 101.0 % of orciprenaline sulfate $[(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4]$, calculated on the dried basis.

Description Orciprenaline Sulfate appears as white crystals or crystalline powder.

Orciprenaline Sulfate is freely soluble in water, slightly soluble in acetic acid (100), and practically insoluble in ether.

A solution of Orciprenaline Sulfate (1 in 20) has no optical rotation.

Melting point—About 220 ºC (with decomposition).

Identification (1) Determine the absorption spectra of solutions of Orciprenaline Sulfate and Orciprenaline Sulfate RS, respectively, in 0.01 mol/L hydrochloric acid TS (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry : both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Orciprenaline Sulfate and Orciprenaline Sulfate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Orciprenaline Sulfate (1 in 100) responds to the Qualitative Tests for sulfate.

pH Dissolve 1.0 g of Orciprenaline Sulfate in 10 mL

of water: the pH of this solution is between 4.0 and 5.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Orciprenaline Sulfate in 10 mL of water: the solution is clear and has no more color than the following control solution.

Control solution—Take 3 mL of Color Matching Fluid T and add 1 mL of diluted hydrochloric acid (1 in 40).

(2) ***Orciprenalone sulfate***—Dissolve 0.200 g of Orciprenaline Sulfate in 0.01 mol/L hydrochloric acid TS to make exactly 20 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry: the absorbance at 328 nm is not more than 0.075.

(3) ***Heavy metals***—Proceed with 2.0 g of Orciprenaline Sulfate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(4) ***Iron***—Dissolve 2.0 g of Orciprenaline Sulfate in 45 mL of water, add 2 mL of hydrochloric acid, and use this solution as the test solution. To 1.0 mL of standard iron solution add water to make 45 mL, add 2 mL of hydrochloric acid, and use this solution as the standard solution. To the test solution and standard solution add 50 mg of ammonium peroxydisulfate and 3 mL of 30 % ammonium thiocyanate solution, and mix: the color of the test solution is not more intense than that of the standard solution (not more than 5 ppm).

(5) ***Related substances***—Dissolve 0.2 g of Orciprenaline Sulfate in a mixture of water and methanol (1 : 5) to make 10 mL, and use this solution as the test solution. Pipet 1 mL of the test solution, add a mixture of water and methanol (1 : 5) to make 100 mL, and use this solution as the standard solution (1). Pipet 5 mL of standard solution (1), add a mixture of water and methanol (1 : 5) to make 10 mL, and use this solution as the standard solution (2). Pipet 5 mL of standard solution (1), add a mixture of water and methanol (1 : 5) to make 20 mL, and use this solution as the standard solution (3). Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and standard solutions (1), (2), and (3) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 2-propanol, water, and ammonia solution (28) (50 : 30 : 16 : 4) to a distance of about 15 cm, and air-dry the plate. Expose the plate to iodine vapor: the spot other than the principal spot obtained form the test solution is not more intense than the principal spot obtained from the standard solution (1) (not more than 1.0 %), and not more than 1 spot is more intense than the principal spot from the standard solution (2) (not more than 0.5 %). The test is not valid if the spot from the standard solution (3) is not clearly visible.

Loss on Drying Not more than 1.5 % (1 g, in vacuum, 105 ºC, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

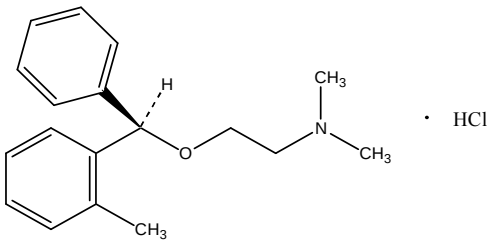
Assay Weigh accurately about 0.7 g of Orciprenaline Sulfate, dissolve in 100 mL of acetic acid (100) by warming in a water-bath and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 52.06 mg of $(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Orphenadrine Hydrochloride



and enantiomer

$C_{18}H_{23}NO \cdot HCl$: 305.84

N,N-Dimethyl-2-[(2-methylphenyl)(phenyl)methoxy] ethanamine hydrochloride [*341-69-5*]

Orphenadrine Hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of orphenadrine hydrochloride ($C_{18}H_{23}NO \cdot HCl$).

Description Orphenadrine Hydrochloride is a white crystalline powder.
Orphenadrine Hydrochloride is freely soluble in water or ethanol (95).

Identification (1) Determine the infrared spectra of Orphenadrine Hydrochloride and Orphenadrine Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) A solution of Orphenadrine Hydrochloride in water (1 in 50) responds to the Qualitative Test (2) for chlorides.

Melting Point About 160 ºC.

Purity (1) ***Clarity and color of solution***—Weigh accurately 0.7 g of Orphenadrine Hydrochloride, and dissolve in ethanol (95) to make exactly 10 mL: it is

clear. Determine the absorbance of this solution as directed under Ultraviolet-visible Spectrophotometry at the wavelength of 436 nm: it is not more than 0.050.

(2) ***Heavy metals***—Proceed with 2.0 g of Orphenadrine Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substance***—Dissolve 0.300 g of Orphenadrine Hydrochloride in water to make 50 mL, add 2 mL of ammonia solution (28) and extract 3 times with each of 10 mL of toluene. Combine the extracts, add anhydrous sodium sulfate, shake, filter and evaporate the filtrate at a temperature not exceeding 50 °C using a rotary evaporator. Dissolve the residue in toluene to make exactly 20 mL and use this solution as the test solution. Separately, dissolve 20 mg of Orphenadrine Hydrochloride RS and 20 mg of Orphenadrine Related Substance I RS {(*RS*)-*N,N*-Dimethyl-2-[(3-methylphenyl) phenylmethoxy]ethanamine} in 20 mL of water, add 1 mL of ammonia solution (28) and extract 3 times with each of 5 mL of toluene. Combine upper layers, add anhydrous sodium sulfate, shake, filter and evaporate the filtrate at a temperature not exceeding 50 °C using a rotary evaporator. Dissolve the residue in toluene to make 20 mL and use this solution as the standard solution. Perform the test with exactly 2 μL of the test solution as directed under Gas Chromatography according to the following conditions, and determine and calculate the percentage of each peak area: any related substance is not more than 0.3 %; total of related substances is not more than 1.0 %. Disregard the peak of related substance not more than 0.02 %.

$$\text{Amount (\%) of related substance} = 100 \times \frac{A_i}{A_S}$$

A_i: Peak area for each related substance obtained from the test solution

A_S: Total peak area of all related substances obtained from the test solution

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A fused-silica column about 0.32 mm in internal diameter and about 60 m in length, coated the inner surface with poly(dimethyl)(diphenyl)siloxane for gas chromatography, 1 μm in thickness.

Split ratio: About 1 : 25

Column temperature: A constant temperature of about 240 ºC.

Injector temperature: A constant temperature of about 290 ºC.

Detector temperature: A constant temperature of about 290 ºC.

Carrier gas: Helium

Flow rate: 1 mL/minute.

System suitability

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the resolution between the peaks of related substance I and orphenadrine is not less than 1.5.

Time span of measurement: About 1.3 times as long as the retention time of orphenadrine beginning after the solvent peak.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Sterility Test It meets the requirement. When the test is applied to the case of using Orphenadrine Hydrochloride in preparation of sterile dosage form without the steriligation process.

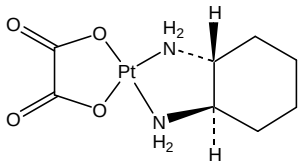
Assay Weigh accurately 0.25 g of Orphenadrine Hydrochloride, previously dried, dissolve in 50 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 30.59 mg of $C_{18}H_{23}NO \cdot HCl$

Containers and Storage ***Containers***—Well-closed or hermetic containers.

Storage—Light-resistant.

Oxaliplatin



$C_8H_{14}N_2O_4Pt$: 397.29

[(1*R*,2*R*)-Cyclohexane-1,2-diamine](ethanedioato-*O,O'*)platinum(II) [*61825-94-3*]

Oxaliplatin contains not less than 98.0 % and not more than 102.0 % of oxaliplatin ($C_8H_{14}N_2O_4Pt$), calculated on the dried basis.

Description Oxaliplatin is a white crystalline powder. Oxaliplatin is slightly soluble in water, very slightly soluble in methanol, and practically insoluble in ethanol (95).

Identification Determine the infrared spectra of Oxaliplatin and Oxaliplatin RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra

exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +74.5 ~ +74.8º (after drying, 0.250 g, water, 50 mL, 100 mm).

Purity (1) ***Clarity and color of solution***—Dissolve 0.10 g of Oxaliplatin in water to make 50 mL: it is colorless and clear.

(2) ***Acidity and alkalinity***—Dissolve 0.10 g of Oxaliplatin in water to make 50 mL, add 0.5 mL of phenolphthalein TS, the resulting solution is colorless. Not more than 0.6 mL of 0.01 mol/L sodium hydroxide VS is required to change the color of the indicator to pink.

(3) ***Related substance***—(i) Related substance I: Weigh accurately 0.10 g of Oxaliplatin, add water, shake well, sonicate very briefly, add water to make 50 mL, and use this solution as the test solution. Separately, weigh accurately 14.0 mg of oxaliplatin related substance I (oxalic acid), dissolve in water to make exactly 250 mL, and use this solution as the standard stock solution. To 5.0 mL of the standard stock solution add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions: the peak area of the related substance I from the test solution is not larger than 3 times the area of the principal peak from the standard solution (0.15 %). Inject the test solution within 20 minutes of preparation.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 205 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with base-deactivated octadecylsilanized silica gel for Liquid Chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 ºC.

Mobile phase: the mixture of pH 6.0 phosphate buffer solution and acetonitrile (8 : 2).

Flow rate: 2 mL/minute

System suitability

System performance: Dissolve 12.5 mg of sodium nitrate in water to make 250 mL. To 2.0 mL of this solution add 25.0 mL of the standard stock solution and water to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating condition, resolution between the peaks of nitrate and related substance I is not less than 9. When the procedure is run with 20 μL of the standard solution under the above operating condition, signal-to-noise ratio for the peak of related substance I is not less than 10.

Time span of measurement: About 2 times as long as the retention time of related substance I beginning after the solvent peak.

pH 6.0 phosphate buffer solution—Dissolve 1.36 g of potassium dihydrogen phosphate in 10 mL of 32 % solution of tetrabutylammonium hydroxide, add water to make 1000 ml, and adjust this solution to pH 6.0 with phosphoric acid.

(ii) Related substance II: Weigh accurately 0.10 g of Oxaliplatin, add water, shake well, sonicate very briefly, add water to make exactly 50 mL, and use this solution as the test solution. Separately, weigh accurately 5 mg of Oxaliplatin Related Substance II [(*SP*-4-2-)-Diaqua[(*1R,2R*)-cyclohexane-1,2-diamine-*κN,κN'*] platinum(diaquodiaminocyclohexane platinum)] RS, dissolve in 25 mL of methanol, add water to make exactly 100.0 mL, sonicate for about 1 hour and 30 minutes until dissolved, and use this solution as the standard stock solution. To 3.0 mL of this solution add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standar solution as directed under Liquid Chromatography according to the following conditions: the peak area of oxaplatin related substance II obtained from the test solution is not larger than 4 times the area of the principal peak from the standard solution (not more than 0.15 %). Inject the test solution within 20 minutes of preparation.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for Liquid Chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 ºC.

Mobile phase: A mixture of pH 3.0 phosphate buffer solution and acetonitrile (8 : 2).

Flow rate: 2 mL/minute

System suitability

System performance: Adjust the pH of 50.0 mL of the standard solution to 6.0 with 0.02 % sodium hydroxide solution. Heat this solution at 70 °C for 4 hours, and cool (generation of related substance V). When the procedure is run with 20 μL of this solution under the above operating conditions, the resolution between the peaks of related substance II and related substance V is not less than 7. When the procedure is run with 20 μL of the standard solution under the above operating conditions, the signal-to-noise ratio of the peak of related substance II is not less than 10.

Relative retention time: The relative retention times of related substance II and related substance V are about 4.3 and 6.4, respectively.

Time span of measurement: About 2.5 times as long as the retention time of related substance II beginning after the solvent peak.

pH 3.0 phosphate buffer solution—To 1.36 g of potassium dihydrogen phosphate and 1 g of sodium

heptanesulfonate add 1000 mL of water to dissolve and adjust to pH 3.0 ± 0.05 with phosphoric acid.

(iii) Related substance III and other related substances: Weigh accurately 0.10 g of Oxaliplatin, add water, shake well, sonicate very briefly to dissolve, add water to make 50 mL, and use this solution as the test solution. Separately, dissolve 5.0 mg of Oxaliplatin Related Substance III {(*OC*-6-33)-[(*1R,2R*)-Cyclohexane-1,2-diamine-$\kappa N,\kappa N'$] [ethanedioato(2-)-$\kappa O^1,\kappa O^2$]dihydroxy-platinum} RS and 5.0 mg of Oxaliplatin RS in water to make exactly 100 mL, and use this solution as the standard solution (1). To 1.0 mL of the standard solution (1) add water to make exactly 100 mL, and use this solution as the standard solution (2). Dissolve 50.0 mg of Oxaliplatin RS in water to make exactly 50 mL, and use this solution as the standard solution (3). Dissolve 5 mg of Dichlorodiamonicyclohexaneplatinum RS in the standard solution (3) to make exactly 50 mL, and use this solution as the standard solution (4). To 5 mL of the standard solution (4) add water to make exactly 50 mL, and use this solution as the standard solution (5). To 0.10 g of Oxaliplatin add 1 mL of the standard solution (1) and water to make exactly 50 mL, and use this solution as the standard solution (6). Perform the test with 10 μL each of the test solution and standard solutions (2), (5), and (6) as directed under Liquid Chromatography according to the following conditions: the peak area of related substance III from the test solution is not larger than 3/4 times the peak area of related substance III from the standard solution (6) (0.15 %), the peak area of any other related substance is not larger than 2 times the peak area of oxaliplatin from the standard solution (2) (0.1 %), and the total area of the peaks is not larger than 3 times the peak area of oxaliplatin from the standard solution (2) (0.15 %). Disregard the peak area of oxaliplatin from the standard solution (2) and any peak with a retention time of less than 2 minutes. Inject the test solution within 20 minutes of preparation.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 ºC.

Mobile phase: A mixture of a solution prepared by mixing 1000 mL of water with 0.6 mL of dilute phosphoric acid and adjusting the pH to 3.0 with sodium hydroxide TS or phosphoric acid, and acetonitrile (99 : 1)

Flow rate: 1.2 mL/minute

System suitability

System performance: When the procedure is run with 10 μL of the standard solution (5) under the above operating conditions, the resolution between the peaks of dichlorodiaminocyclohexaneplatinum and oxaliplatin is not less than 2. When the procedure is run with 10 μL of the standard solution (2) under the above operating conditions, the signal-to-noise ratios of the peaks of related substance III and oxaliplatin are not less than 50 and not less than 10, respectively.

Time span of measurement: About 3 times as long as the retention time of oxaliplatin beginning after the solvent peak.

(iv) Total related substances: The sum of related substances I, II, III, and other related substances is not greater than 0.30 %.

(4) ***Related substance IV***—Weigh accurately 30 mg of Oxaliplatin, dissolve in methanol to make 50 mL and use this solution as the test solution. Dissolve 5 mg of Oxaliplatin Related Substance IV {(*SP*-4-2)-[(*1S,2S*)-Cyclohexane-1,2-diamine-$\kappa N,\kappa N'$] [ethanediato(2-)-$\kappa O^1,\kappa O^2$]platinum} RS in methanol to make exactly 100 mL and use this solution as the standard solution (1). Pipet 15.0 mL of the standard solution (1), add methanol to make exactly 50 mL and use this solution as the standard solution (2). Dissolve 75 mg of Oxaliplatin RS in methanol to make exactly 100 mL and use this solution as the standard solution (3). Dilute 5.0 mL of the standard solution (3) to exactly 100 mL with methanol and use this solution as the standard solution (4). To 40 mL of the standard solution (3) add 1.0 mL of the standard solution (1) and methanol to make exactly 50 mL and use this solution as the standard solution (5). To 4.0 mL of the standard solution (1) add 5.0 mL of the standard solution (4) and methanol to make exactly 50 mL and use this solution as the standard solution (6). Perform the test with exactly 20 μL each of the test solution and standard solutions (5) and (6) as directed under Liquid Chromatography according to the following conditions, the peak height of related substance IV from the test solution is not more than 3 times that of related substance IV with the standard solution (5) (0.15 %).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with silica gel OC for chiral separations for liquid chromatography.

Column temperature: A constant temperature of about 40 ºC.

Mobile phase: A mixture of methanol and ethanol (99.5) (7 : 3)

Flow rate: 0.3 mL/minute.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution (6) under the above operating conditions, the resolution between the peaks of oxaliplatin and related substance IV is not less than 1.5. When the procedure is run with 20 μL of the

standard solution (5) under the above operating condition, signal-to-noise ratio is not less than 10 for the peak of related substance IV.

Time span of measurement: About 2 times as long as the retention time of Oxaliplatin beginning after the solvent peak.

(5) ***Silver***—Weigh accurately 0.1000 g of Oxaliplatin, dissolve in water to make exactly 50 mL. Pipet 20 μL of this solution, add 0.5 mol/L nitric acid TS to make exactly 40 μL and use this solution as the test solution. Separately, weigh accurately about 1.575 g of silver nitrate, dissolve in 0.5 mol/L nitric acid TS to make exactly 1000 mL. Dilute adequate amount of this solution with 0.5 mol/L nitric acid TS to make a solution containing 10 ppb silver per each mL and use this solution as the standard solution (1). To 20 μL of the test solution add 8 μL of the standard solution (1) and 0.5 mol/L nitric acid to make 40 μL and use this solution as the standard solution (2). To 20 μL of the test solution add 16 μL of the standard solution (1) and 0.5 mol/L nitric acid to make 40 μL and use this solution as the standard solution (3). Perform the test with the test solution and standard solutions (2) and (3) as directed in the standard addition method under the Atomic Absorption Spectrophotometry according to the following conditions and calculate the concentration of silver in the test solution (not more than 5 ppm).

Gas: Dissolved acetylene or hydrogen - Air
Lamp: A silver hollow cathode lamp.
Wavelength: 328.1 nm.

Loss on Drying Not more than 0.5 % (1.0 g, 105 °C, 2 hours).

Bacterial Endotoxins Less than 1.0 EU/mg (when Oxaliplatin is used in a sterile preparation without a further appropriate procedure for the removal of bacterial endotoxins).

Assay Weigh accurately about 50 mg each of Oxaliplatin and oxaliplatin RS, dissolve in water to make 500 mL and use the solutions as the test solution and the standard solution, respectively. Perform the test with with 20 μL each of the test solution and the standard solution as directed in Related substance III and other related substances of Purity according to the above operating condition and calculate the peak area of oxaliplatin, A_T and A_S, respectively.

$$\text{Amount (mg) of oxaliplatin } (C_8H_{14}N_2O_4Pt) = 500 \times C \times \frac{A_T}{A_S}$$

C: Concentration (mg/mL) of Oxaliplatin RS in the standard solution.

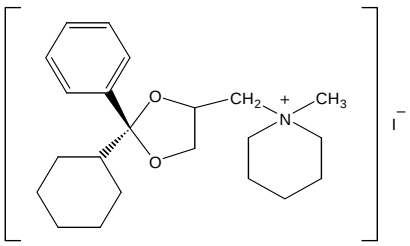
Operation conditions
System suitability

System performance: Weigh 5 mg of dichlorodiaminocyclohexaneplatinum RS, add a solution, prepared by dissolving 50 mg of Oxaliplatin RS in water to make 500 mL, to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating condition, the resolution between the peaks of dichlorodiaminocyclohexaneplatinum and oxaliplatin is not less than 2.0.

System repeatability: When the test is repeated 5 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of oxaliplatin is not more than 2.0 %.

Containers and Storage ***Containers***—Hermetic containers (when the label states that it contains no bacterial endotoxins).

Oxapium Iodide



and enantiomer

$C_{22}H_{34}INO_2$: 471.42

1-[(2-Cyclohexyl-2-phenyl-1,3-dioxolan-4-yl)methyl]-1-methylpiperidin-1-ium iodide [*6577-41-9*]

Oxapium Iodide, when dried, contains not less than 98.5 % and not more than 101.0 % of oxapium iodide ($C_{22}H_{34}INO_2$).

Description Oxapium Iodide appears as white, crystalline powder.
Oxapium Iodide is soluble in methanol, in ethanol (95), or in acetonitrile, slightly soluble in water, in acetic anhydride, or in acetic acid (100), and practically insoluble in ether.
A solution of Oxapium Iodide in methanol (1 in 100) has no optical rotations.

Identification (1) Dissolve 0.1 g of Oxapium Iodide in 10 mL of methanol and add 2 mL of dilute nitric acid and 2 mL of silver nitrate TS: a greenish yellow precipitate is produced.

(2) Determine the infrared spectra of Oxapium Iodide and Oxapium Iodide RS, previously dried, as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting Point 198 ~ 203 °C.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Oxapium Iodide according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Dissolve 50 mg of Oxapium Iodide in 100 mL of a mixture of water and acetonitrile (1 : 1) and use this solution as the test solution. Pipet 1 mL of the test solution, add a mixture of water and acetonitrile (1 : 1) to make exactly 50 mL and use this solution as the standard solution. Perform the test with 50 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine each peak area of each solution by the automatic integration method: the total area of all peaks other than the principal peak of the test solution is not greater than the area of the principal peak from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature between 20 °C and 30 °C.

Mobile phase: To 57 mL of acetic acid (100) and 139 mL of triethylamine, add water to make 1000 mL. To 50 mL of this solution, add 500 mL of acetonitrile, 10 mL of dilute acetic acid and 440 mL of water.

Flow rate: Adjust the flow rate so that the retention time of Oxapium is about 4 minutes.

System suitability

Detection sensitivity: Adjust the detection sensitivity so that the peak height of oxapium obtained from 50 μL of the standard solution composes 5 % to 15 % of the full scale.

System performance: Dissolve 50 mg of Oxapium Iodide and 3 mg of benzophenone in 100 mL of the mobile phase. When the procedure is run with 20 μL of this solution according to the above operating conditions, oxapium and benzophenone are eluted in this order with the resolution between their peaks being not less than 5.

Time span of measurement: About 6 times as long as the retention time of oxapium after the peak of iodide ion.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

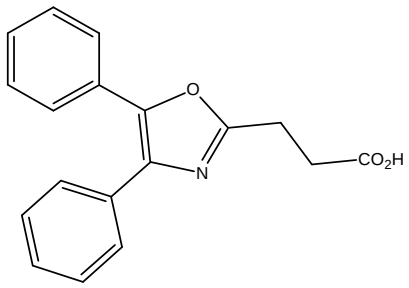
Assay Weigh accurately about 0.7 g of Oxapium Iodide, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (9 : 1) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 47.14 mg of $C_{22}H_{34}INO_2$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Oxaprozin



$C_{18}H_{15}NO_3$: 292.32

3-(4,5-Diphenyl-1,3-oxazol-2-yl)propanoic acid
[*82419-36-1*]

Oxaprozin, when dried, contains not less than 98.5 % and not more than 101.0 % of oxaprozin ($C_{18}H_{15}NO_3$).

Description Oxaprozin appears as white to yellowish white crystalline powder.
Oxaprozin is sparingly soluble in methanol or in ethanol (95), slightly soluble in ether, and practically insoluble in water.
Oxaprozin is gradually affected colored by light.

Melting point—About 265 °C (with decomposition).

Identification Determine the infrared spectra of Oxaprozin and Oxaprozin RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 161 ~ 165 °C.

Absorbance $E_{1\,cm}^{1\%}$ (285 nm): 455 ~ 495 (after drying, 10 mg, methanol, 1000 mL).

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Oxaprozin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) ***Arsenic***—Prepare the test solution with 2.0 g of Oxaprozin according to Method 3, and perform the test (not more than 1 ppm).

(3) ***Related substances***—Dissolve 0.1 g of Oxaprozin in 10 mL of methanol, and use this solution as the test solution. Pipet 1 mL of the test solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 5 mL, 3 mL, and 1 mL of the standard solution, add methanol to each to make exactly 10 mL, and use these solutions as the standard solutions (2), (3) and (4), respectively. Perform the test with these solutions as directed under Thin Layer Chromatography. Spot 10 μL each of the test solution and standard solutions (1), (2), (3) and (4) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and acetic acid (100) (99:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the total intensity of all spots other than the principal spot from the test solution is not more than 1.0 % calculated on the basis of intensities of the spots from the standard solutions (1), (2), (3) and (4).

Loss on Drying Not more than 0.3 % (1 g, 105 ºC 2 hours)

Residue on Ignition Not more than 0.3 % (1 g).

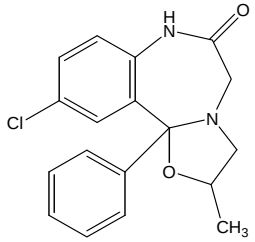
Assay Weigh accurately about 0.5 g of Oxaprozin, previously dried, dissolve in 50 mL of ethanol (95), and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 29.33 mg of $C_{18}H_{15}NO_3$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Oxazolam



$C_{18}H_{17}ClN_2O_2$: 328.79

10-Chloro-2-methyl-11b-phenyl-2,3,7,11b-tetrahydrobenzo[f]oxazolo[3,2-d][1,4]diazepin-6(5*H*)-one [*24143-17-7*]

Oxazolam, when dried, contains not less than 99.0 % and not more than 101.0 % of oxazolam ($C_{18}H_{17}ClN_2O_2$).

Description Oxazolam appears as white crystals or crystalline powder, is odorless and tasteless.
Oxazolam is freely soluble in glacial acetic acid, soluble in 1,4-dioxane or in dichloromethane, slightly soluble in ethanol (95) or in ether, and practically insoluble in water.
Oxazolam dissolves in dilute hydrochloric acid.
Oxazolam is gradually colored by light.

Melting point—About 187 °C (with decomposition).

Identification (1) Dissolve 10 mg of Oxazolam in 10 mL of ethanol (95) by heating and add 1 drop of hydrochloric acid: a pale yellow color is observed and the solution shows a yellow-green fluorescence under ultraviolet light (main wavelength: 365 nm). Add 1 mL of sodium hydroxide TS to this solution: the color and fluorescence of this solution disappear immediately.

(2) Dissolve 10 mg of Oxazolam in 5 mL of dilute hydrochloric acid by heating in a water-bath for 10 minutes. After cooling, 1 mL of this solution responds to the Qualitative Tests for primary aromatic amines.

(3) Place 2 g of Oxazolam in a flask, add 50 mL of ethanol (95) and 25 mL of 6 mol/L hydrochloric acid TS and boil under a reflux condenser for 5 hours. After cooling, neutralize with a solution of sodium hydroxide (1 in 4) and extract with 30 mL of dichloromethane. Dehydrate with 3 g of anhydrous sodium sulfate, filter and evaporate the dichloromethane of the filtrate. Dissolve the residue in 20 mL of methanol by heating in a water-bath and cool immediately in an ice-bath. Collect the crystals and dry in vacuum at 60 °C for 1 hour: the crystals melt between 96 °C and 100 °C.

(4) Determine the absorption spectra of solutions of Oxazolam and Oxazolam RS, respectively, in ethanol (95) (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry : both spectra exhibit similar intensities of absorption at the same wavelengths.

(5) Proceed with Oxazolam as directed under the Flame Coloration Test (2) and perform the test: a green color is observed.

Absorbance $E_{1\,cm}^{1\%}$ (246 nm): 410 ~ 430 (after drying, 1 mg, ethanol (95), 100 mL).

Purity (1) ***Chloride***—Take 1.0 g of Oxazolam, add 50 mL of water, allow to stand for 1 hour with occasional shaking and filter. To 25 mL of this filtrate, add 6 mL of dilute nitric acid and water to make 50 mL and perform the test. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014 %).

(2) ***Heavy metals***—Proceed with 1.0 g of Oxazolam according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Place 1.0 g of Oxazolam in a Kjedahl flask, add 5 mL of sulfuric acid and 5 mL of nitric acid

and heat gently. Repeat the addition of 2 mL to 3 mL of nitric acid at times and continue to heat until a colorless to pale yellow solution is obtained. After cooling, add 15 mL of saturated ammonium oxalate solution, heat the solution until dense white fumes are evolved and evaporate to a volume of 2 mL to 3 mL. After cooling, dilute with water to make 10 mL and perform the test with this solution as the test solution (not more than 2 ppm).

(4) ***Related substances***—Dissolve 50 mg of Oxazolam in 10 mL of dichloromethane and use this solution as the test solution. Pipet 1 mL of this solution, add dichloromethane to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Immediately air-dry, develop the plate with a mixture of toluene and acetone (8 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (wavelength: 254 nm): any spot other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

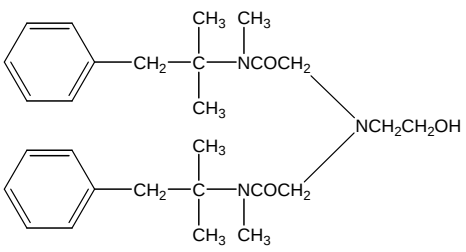
Assay Weigh accurately about 0.65 g of Oxazolam, previously dried and dissolve in 100 mL of a mixture of acetic acid (100) and 1,4-dioxane (1 : 1). Titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 2 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.879 mg of $C_{18}H_{17}ClN_2O_2$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Oxethazaine



$C_{28}H_{41}N_3O_3$: 467.64

2,2'-[(2-Hydroxyethyl)imino]*bis*[*N*-methyl-*N*-(2-methyl-1-phenyl-2-propanyl)acetamide] [*126-27-2*]

Oxethazaine, when dried, contains not less than 98.5 % and not more than 101.0 % of oxethazaine ($C_{28}H_{41}N_3O_3$).

Description Oxethazaine appears as white to pale yellowish white, crystalline power.
Oxethazaine is very soluble in acetic acid (100), freely soluble in methanol or ethanol (95), sparingly soluble in ether and practically insoluble in water.

Identification (1) Determine the absorption spectra of the solutions of Oxethazaine and Oxethazaine RS, respectively, in ethanol (95) (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Oxethazaine and Oxethazaine RS, respectively, as directed in the potassium bromide disk method under Infrared Spectrophotometry; both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 101 ~ 104 °C

Purity (1) ***Chloride***—Dissolve 1.0 g of Oxethazaine in 20 mL of ethanol (95), add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS, 20 mL of ethanol (95), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.011 %).

(2) ***Heavy metals***—Proceed with 2.0 g of Oxethazaine according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Dissolve 0.40 g of Oxethazaine in 10 mL of ethanol (95) and use this solution as the test solution. Pipet 1 mL of the test solution, add ethanol (95) to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of isopropylether, tetrahydrofuran, methanol and ammonia solution (28) (24 : 10 : 5 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

(4) ***2-Aminoethanol***—Take 1.0 g of Oxethazaine, add methanol to make exactly 10 mL, then add 0.1 mL of a solution of 1-fluoro-2,4-dinitrobenzene in methanol (1 in 25), shake well and heat at 60 °C for 20

minutes: the solution has no more color than the following control solution.

Control solution—Take 0.10 g of 2-aminoethanol, add methanol to make exactly 200 mL, pipet 1.0 mL of this solution and add methanol to make exactly 10 mL. Proceed as directed above.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, 60 °C, 3 hours).

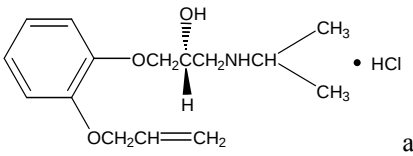
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.9 g of Oxethazaine, previously dried, dissolve in 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (indicator: 2 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 46.76 mg of $C_{28}H_{41}N_3O_3$

Containers and Storage ***Containers***—Tight containers.

Oxprenolol Hydrochloride



and enantiomer

$C_{15}H_{23}NO_3 \cdot HCl$: 301.81

1-(Propan-2-ylamino)-3-(2-prop-2-enoxyphenoxy)propan-2-ol hydrochloride [*6452-73-9*]

Oxprenolol Hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of oxprenolol hydrochloride ($C_{15}H_{23}NO_3 \cdot HCl$).

Description Oxprenolol Hydrochloride is a white, crystalline powder.
Oxprenolol Hydrochloride is very soluble in water, freely soluble in ethanol (95) or in acetic acid (100), slightly soluble in acetic anhydride, and practically insoluble in ether.

Identification (1) Take 2 mL of a solution of Oxprenolol Hydrochloride (1 in 100) and add 1 drop of copper (II) sulfate TS and 2 mL of sodium hydroxide TS: a blue-purple color is observed. To this solution, add 1 mL of ether, shake well and allow to stand: a red-purple color is observed in the ether layer and a blue-purple color is observed in the water layer.

(2) Take 3 mL of a solution of Oxprenolol Hydrochloride (1 in 150) and add 3 drops of Reinecke salt TS: a pale red precipitate is produced.

(3) Determine the infrared spectra of Oxprenolol Hydrochloride and Oxprenolol Hydrochloride RS, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(4) A solution of Oxprenolol Hydrochloride (1 in 50) responds to the Qualitative Tests for chloride.

Melting Point 107 ~ 110 °C.

pH Dissolve 1.0 g of Oxprenolol Hydrochloride in 10 mL of water: the pH of this solution is between 4.5 and 6.0.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Oxprenolol Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 2.0 g of Oxprenolol Hydrochloride according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Oxprenolol Hydrochloride according to Method 3 and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 0.25 g of Oxprenolol Hydrochloride in 10 mL of water and use this solution as the test solution. Pipet 4 mL of the test solution and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate in a developing chamber saturated with ammonia vapor with a mixture of chloroform and methanol (9 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot from the test solution is not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 80 °C, 3 hours).

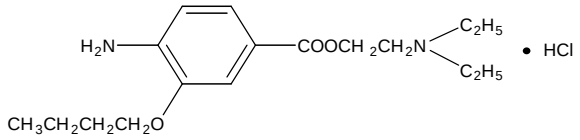
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.6 g of Oxprenolol Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 30.181 mg of $C_{15}H_{23}NO_3 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Oxybuprocaine Hydrochloride



Benoxinate Hydrochloride

$C_{17}H_{28}N_2O_3 \cdot HCl$: 344.88

2-(Diethylamino)ethyl 4-amino-3-butoxybenzoate hydrochloride [*5987-82-6*]

Oxybuprocaine Hydrochloride, when dried, contains not less than 99.0 % and not more than 101.0 % of oxybuprocaine hydrochloride ($C_{17}H_{28}N_2O_3 \cdot HCl$).

Description Oxybuprocaine Hydrochloride appears as white crystals or crystalline powder, is odorless, has a saline taste, and exhibits an anesthetic action when placed on the tongue.
Oxybuprocaine Hydrochloride is very soluble in water, freely soluble in ethanol (95) or in chloroform, and practically insoluble in ether.
Oxybuprocaine Hydrochloride is colored by light.

pH—The pH of a solution of Oxybuprocaine Hydrochloride (1 in 10) is between 5.0 and 6.0.

Identification (1) Dissolve 10 mg of Oxybuprocaine Hydrochloride in 1 mL of dilute hydrochloric acid and 4 mL of water. This solution responds to the Qualitative Tests for primary aromatic amines.

(2) Dissolve 0.1 g of Oxybuprocaine Hydrochloride in 8 mL of water and add 3 mL of ammonium thiocyanate TS: an oily substance is produced. Rub the inner surface of the container with a glass rod: white crystals are formed. Collect the crystals so obtained, recrystallize from water and dry in a desiccator (in vacuum, P_2O_5) for 5 hours: the crystals melt between 103 °C and 106 °C.

(3) Determine the absorption spectra of solutions of Oxybuprocaine Hydrochloride and Oxybuprocaine Hydrochloride RS, respectively, in water (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry : both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) A solution of Oxybuprocaine Hydrochloride (1 in 10) responds to the Qualitative Tests for chloride.

Melting Point 158 ~ 162 °C.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Oxybuprocaine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Oxybuprocaine Hydrochloride according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Dissolve 0.25 g of Oxybuprocaine Hydrochloride in 10 mL of chloroform and use this solution as the test solution. Pipet 1 mL of the test solution and add chloroform to make exactly 20 mL. Pipet 1 mL of this solution, add chloroform to make exactly 50 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, ethanol (95) and formic acid (7 : 2 : 1) to a distance of about 10 cm and air-dry the plate. Spray evenly *p*-dimethylaminobenzaldehyde TS for spraying on the plate: any spot other than the principal spot from the test solution is not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

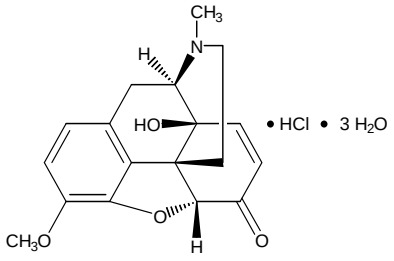
Assay Weigh accurately about 0.6 g of Oxybuprocaine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 34.488 mg of $C_{17}H_{28}N_2O_3 \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Oxycodone Hydrochloride Hydrate



$C_{18}H_{21}NO_4 \cdot HCl \cdot 3H_2O$: 405.87

(1*S*,5*R*,13*R*,17*S*)-17-Hydroxy-10-methoxy-4-methyl-12-oxa-4-azapentacyclo[9.6.1.0^{1,13}.0^

{5,17}.0^{7,18}]octadeca-7(18),8,10-trien-14-one trihydrate hydrochloride [*591229-40-2*]

Oxycodone Hydrochloride Hydrate contains not less than 98.0 % and not more than 101.0 % of oxycodone hydrochloride ($C_{18}H_{21}NO_4 \cdot HCl$: 351.83), calculated on the anhydrous basis.

Description Oxycodone Hydrochloride Hydrate is a white, crystalline powder.
Oxycodone Hydrochloride Hydrate is freely soluble in water, methanol or acetic acid (100), sparingly soluble in ethanol (95), slightly soluble in acetic anhydride, and practically insoluble in ether.
The pH of a solution dissolved 1.0 g of Oxycodone Hydrochloride Hydrate in 10 mL of water is between 3.8 and 5.8.
Oxycodone Hydrochloride Hydrate is colored by light.

Identification (1) Determine the absorption spectra of solutions of Oxycodone Hydrochloride Hydrate and Oxycodone Hydrochloride Hydrate RS, respectively, in water (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Oxycodone Hydrochloride and Oxycodone Hydrochloride Hydrate RS, respectively, as directed in the potassium bromide disk method under Infrared Spectrophotometry; both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Oxycodone Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests (2) for chloride.

Specific Optical Rotation $[\alpha]_D^{20}$: -140 ~ -149° (0.5 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Oxycodone Hydrochloride Hydrate in 10 mL of water: the solution is clear and colorless.

(2) ***Morphine***—Dissolve 10 mg of Oxycodone Hydrochloride Hydrate in 1 mL of water, add 5 mL of 1-nitroso-2-naphthol TS and 2 mL of a solution of potassium nitrate (1 in 10) and warm 40 °C for 2 minutes. To this solution, add 1 mL of a solution of sodium nitrite (1 in 5000) and warm at 40 °C for 5 minutes. After cooling, add 10 mL of chloroform, shake, centrifuge and separate the water layer: the color of the solution is not more intense than a pale red.

(3) ***Codeine***—Dissolve 10 mg of Oxycodone Hydrochloride Hydrate in 5 mL of sulfuric acid, add 1 drop of iron (III) chloride TS and warm: no blue color is observed. Add 1 drop of nitric acid: no red color is observed.

(4) ***Thebaine***—Dissolve 0.1 g of Oxycodone Hydrochloride Hydrate in 2 mL of diluted hydrochloric acid (1 in 10) and heat the solution in a water-bath for 25 minutes. After cooling, add 0.5 mL of 4-aminoantipyrine hydrochloride TS and 0.5 mL of a solution of potassium hexacyanoferrate (III) (1 in 100) and shake. Then shake the solution with 2 mL of ammonia TS and 3 mL of chloroform: no red color is observed in the chloroform layer.

Water 12 ~ 15 % (0.2 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (0.5 g).

Assay Weigh accurately about 0.5 g of Oxycodone Hydrochloride Hydrate, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 35.183mg of $C_{18}H_{21}NO_4 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.
Storage—Light-resistant.

Oxygen

O_2: 32.00

[*7782-44-7*]

Oxygen contains not less than 99.5 vol % and not more than 101.0 vol % of oxygen (O_2).

Description Oxygen is a colorless gas and is odorless.
1 mL of Oxygen dissolves in 32 mL of water and in 7 mL of ethanol at 20 °C and at a pressure of 101.3 kPa.
1000 mL of Oxygen at 0 °C and at 101.3 kPa weighs about 1.429 g.

Identification (1) Put a glowing splinter of wood into Oxygen: it bursts into flame immediately.

(2) Transfer 1 mL each of Oxygen and oxygen directly from metal cylinders with a pressure-reducing valve to gas-measuring tubes or syringes for gas chromatography, using a polyvinyl chloride induction tube. Perform the test with these gases as directed under Gas Chromatography according to the conditions of the Purity (2): the retention time of principal peak from Oxygen coincides with that of oxygen.

Purity Keep the containers of Oxygen between 18 °C and 22 °C for not less than 6 hours before carrying out the following tests and calculate the volume to be used with reference to the gas at 20 °C and at 101.3 kPa.

(1) ***Acid or alkali, carbon dioxide, oxidizing substances and chloride***—Perform the test under the conditions of purity (1), (2), (3) and (5) of Nitrous Oxide.

(2) ***Nitrogen***—Introduce 1.0 mL of Oxygen into a gas-measuring tube or syringe for gas chromatography from a metal hermetic container under pressure through a pressure-reducing valve and a directly connected polyvinyl tube. Perform the test as directed under Gas Chromatography according to the following conditions and determine the peak area, A_T, of nitrogen. Introduce 0.50 mL of nitrogen into the gas mixer, draw carrier gas into the mixer to make exactly 100 mL and allow to mix thoroughly. Perform the test in the same manner with 1.0 mL of this mixture as directed above and determine the peak area, A_S of nitrogen: A_T is not larger than A_S.

Operating conditions

Detector: A thermal conductivity detector.

Column: A column, about 3 mm in internal diameter and about 3 m in length, packed with 250 μm to 355 μm zeolite (0.5 mm pore size)for gas chromatography.

Column temperature: A constant temperature of about 50 °C.

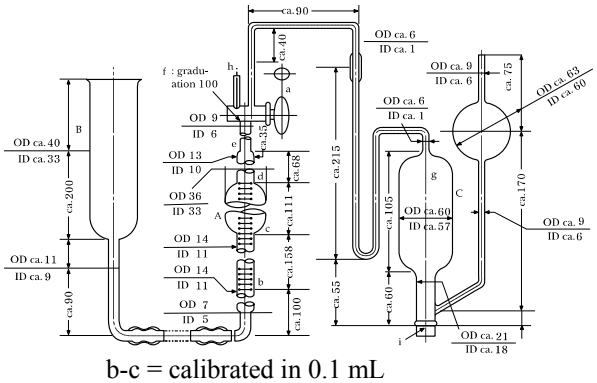
Carrier gas: Hydrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of nitrogen is about 5 minutes.

Selection of column: Introduce 0.5 mL of nitrogen into a gas mixer, add Oxygen to make 100 mL and mix well. When the procedure is run with 1.0 mL of the mixture under the above operating conditions, use a column giving elution of oxygen and nitrogen in this order with well-resolved peaks.

(3) ***Carbon monoxide***—Proceed in the same manner as directed in the Purity (6) Nitrogen dioxide under Carbon Dioxide. Pass 1000 ± 50 mL of Oxygen in the gas phase through a carbon monoxide detector tube at the constant rate, and determine the amount of carbon monoxide: not more than 0.001 %.

Assay (1) ***Apparatus***—The apparatus is shown diagrammatically in the accompanying figure. A is a 100-mL gas buret having a two-way stopcock, a, b-c, d-e and e-f are graduated in 0.1 mL and c-d is graduated in 2 mL. A is properly connected with a leveling tube, B by a thick rubber tube. Fill ammonium chloride-ammonia TS up to the middle of A and B. Place in the absorption ball, g of the gas pipette, C, a coil of copper wire, not more than 2 mm in diameter, which extends to the uppermost portion of the bulb, add 125 mL of ammonium chloride-ammonia TS and stopper with a rubber stopper, I. Connect C with A using the thick rubber tube.



b-c = calibrated in 0.1 mL
c-d = calibrated in 2 mL
d-e = calibrated in 0.1 mL
e-f = calibrated in 0.1 mL
the graduations are marked with red line.
b-f = calibrated in 100 mL

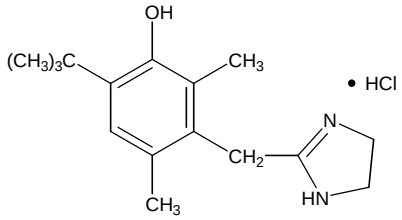
(2) ***Procedure***—Open a, set B downward and draw the liquid in g to the stopcock opening a. Then close a. Open a to the intake tube h and fill A and h with ammonium chloride-ammonia TS by lifting B. Close a, connect h with a container of Oxygen, open a, set B downward and measure accurately 100 mL of Oxygen. Open a toward C and transfer the Oxygen to g by lifting B. Close a and rock C gently for 5 minutes. Open a, draw the residual gas back into A by setting B downward and measure the volume of the residual gas. Repeat the procedure until the volume of residual gas is constant and designate this as V (mL). With fresh ammonium chloride-ammonia TS in C, repeat the procedure at least 4 times and measure the volume of residual gas. Calculate V and the volume of Oxygen used as the sample with reference to the gas at 20 °C and at 101.3 kPa.

Volume (mL) of Oxygen (O_2) = calculated volume of the sample (mL) - calculated volume of V (mL)

Containers and Storage ***Containers***—Metal cylinders.

Storage—Not exceeding 40 °C.

Oxymetazoline Hydrochloride



$C_{16}H_{24}N_2O \cdot HCl$: 296.84

6-*tert*-Butyl-3-(4,5-dihydro-1*H*-imidazol-2-ylmethyl)-2,4-dimethylphenol hydrochloride [*2315-02-8*]

Oxymetazoline Hydrochloride contains not less than 98.5 % and not more than 101.5 % of oxymetazoline hydrochloride ($C_{16}H_{24}N_2O \cdot HCl$), calculated on the dried basis.

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Description Oxymetazoline Hydrochloride appears as white, fine crystalline powder

Oxymetazoline Hydrochloride is soluble in water or in ethanol (95) and practically insoluble in benzene, in chloroform or in ether.

Oxymetazoline Hydrochloride is hygroscopic.

Melting point—About 300 °C (with decomposition).

Identification (1) Determine the absorption spectra of solutions of Oxymetazoline Hydrochloride and Oxymethazoline Hydrochloride RS in ethanol (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths, and the difference of each absorbance of Oxymetazoline Hydrochloride and Oxymetazoline Hydrochloride RS at the wavelength of a maximum absorbance at about 279 nm is not more than 3.0 %.

(2) Determine the infrared spectra of Oxymetazoline Hydrochloride and Oxymetazoline Hydrochloride RS, previously dried, as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit maxima at the same wave numbers.

(3) Take a solution of about 50 mg in 3 mL of water and add 1 mL of ammonium hydroxide TS, filter and acidify the filtrate with nitric acid: the filtrate responds to the Qualitative Tests for chloride.

pH The pH of the solution containing 1.0 g of Oxymetazoline Hydrochloride in 20 mL of water is between 4.0 and 6.5.

Purity ***Heavy metals***—Proceed with 1.0 g of Oxymetazoline Hydrochloride according to the Method 2 and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

Loss on Drying Not more than 1.0 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Transfer about 25 mg of Oxymetazoline Hydrochloride, accurately weighed, to a volumetric flask, dissolve in mobile phase, dilute with mobile phase to make 50 mL and mix. Use this solution as the test solution. Prepare a solution in mobile phase of Oxymetazoline Hydrochloride RS, previously dried at 105 °C for 3 hours, having a known concentration of about 0.5 mg per mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution, as directed under Liquid Chromatography. Measure the peak areas, A_T and A_S, of oxymetazoline Hydrochloride for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of oxymetazoline hydrochloride } (C_{16}H_{24}N_2O \cdot HCl) = 50 \times C \times \frac{A_T}{A_S}$$

C: Concentration (mg/mL) of Oxymetazoline Hydrochloride in the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with multiple pore (strong acid cationic ion exchange resin) silica gel for liquid chromatography.

Mobile phase: A mixture of water, methanol, 1 mol/L sodium acetic acid and acetic acid (100) (46 : 40 : 10 : 4).

Flow rate: 1 mL/minute.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution, the symmetry factor is not more than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of oxymetazoline is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Oxymetazoline Hydrochloride Nasal Solution

Oxymetazoline Hydrochloride Nasal Solution contains not less than 90.0 % and not more than 110.0 % of the labeled amount of oxymetazoline hydrochloride ($C_{16}H_{24}N_2O \cdot HCl$: 296.84).

Method of Preparation Prepare as directed under Nasal Solution, with Oxymetazoline Hydrochloride.

Description Oxymetazoline Hydrochloride Nasal Solution is a clear and colorless liquid.

Identification Place a volume of Oxymetazoline Hydrochloride Nasal Solution, equivalent to about 2.5 mg of Oxymetazoline Hydrochloride, in a separatory funnel and add water to make about 10 mL. Add 2 mL of sodium carbonate solution (1 in 10), extract with 10 mL of chloroform and transfer the chloroform solution with 10 mL of 0.1 mol/L hydrochloric acid, allow to separate and discard the chloroform layer. Transfer 8 mL of the acidic aqueous layer to a test tube, neutralize

by the drop-wise addition of 1 mol/L sodium hydroxide, add 1 drop of 1 mol/L sodium hydroxide in excess and mix. Add a few drops of sodium pentacyanonitrosylferrate (III) TS and 2 drops of sodium hydroxide solution (15 in 100), mix and allow to stand for 10 minutes. Add 0.1 mol/L hydrochloric acid drop-wise until the pH is between 8 and 9 and allow to stand for 10 minutes: a purple color is observed.

pH 4.0 ~ 6.5.

Assay Use Oxymetazoline Hydrochloride Nasal Solution as the test solution. Separately, weigh accurately a portion of Oxymetazoline Hydrochloride RS, previously dried at 105 °C for 3 hours, dissolve in the mobile phase having a known concentration, approximately equal to the labeled concentration of the Oxymetazoline Hydrochloride Nasal Solution. Use this solution as the standard solution. Perform the test with the test solution and the standard solution, as directed in the Assay under Oxymetazoline Hydrochloride.

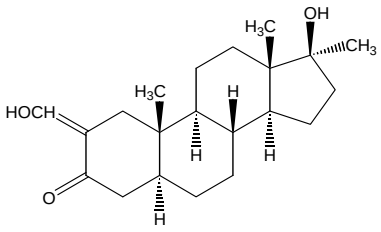
Amount (mg/mL) of oxymetazoline hydrochloride

$$(C_{16}H_{24}N_2O\cdot HCl) = C \times \frac{A_T}{A_S}$$

C: Concentration (mg/mL) of Oxymetazoline Hydrochloride in the standard solution.

Containers and Storage ***Containers***—Tight containers.

Oxymetholone



$C_{21}H_{32}O_3$: 332.48

17*β*-Hydroxy-2-hydroxymethylidene-17*α*-methyl-3-androstanone [*434-07-1*]

Oxymetholone, when dried, contains not less than 97.0 % and not more than 103.0 % of oxymetholone ($C_{21}H_{32}O_3$).

Description Oxymetholone is a white to pale yellowish white, crystalline powder and is odorless.
Oxymetholone is freely soluble in chloroform, soluble in 1,4-dioxane, sparingly soluble in methanol, in ethanol (95) or in acetone, slightly soluble in ether, and practically insoluble in water.
Oxymetholone is gradually colored and decomposed by light.

Identification (1) Dissolve 2 mg of Oxymetholone in 1 mL of ethanol (95) and add 1 drop of iron (III) chloride TS: a purple color is observed.

(2) Dissolve 10 mg each of Oxymetholone and Oxymetholone RS in methanol to make 50 mL. To 5 mL each of the solutions, add 5 mL of sodium hydroxide-methanol TS and methanol to make 50 mL. Determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry, respectively : both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Oxymetholone and Oxymetholone RS, respectively, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +34 ~ +38° (0.2 g after drying, 1,4-dioxane, 10 mL, 100 mm).

Melting Point 175 ~ 182 °C

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Oxymetholone in 25 mL of 1,4-dioxane: the solution is clear and shows colorless to a pale yellow color.

(2) ***Related substances***—Dissolve 50 mg of Oxymetholone in 5 mL of chloroform and use this solution as the test solution. Pipet 1 mL of the test solution, add chloroform to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography and air-dry the spot. Develop immediately the plate with a mixture of toluene and ethanol (99.5) (49 : 1) to a distance of about 12 cm and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate and heat at 100 °C for 3 minutes to 5 minutes: any spot other than the principal spot and starting point obtained from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 1.0 % (0.5 g, in vacuum, P_2O_5, 4 hours).

Residue on Ignition Not more than 0.15 % (0.5 g).

Assay Weigh accurately about 40 mg of Oxymetholone, previously dried, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution and add methanol to make exactly 50 mL. To exactly measured 5 mL of this solution, add 5 mL of sodium hydroxide-methanol TS and methanol to make exactly 50 mL. Determine the absorbance, *A,* of this solution at the wavelength of a maximum absorption at about 315 nm, as directed under Ultraviolet-visible

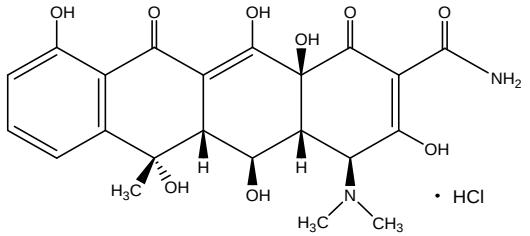
Spectrophotometry using a solution, prepared by adding methanol to 5 mL of sodium hydroxide-methanol TS to make 50 mL, as the blank.

$$\text{Amount (mg) of Oxymetholone } (C_{21}H_{32}O_3) = \frac{A}{541} \times 50000$$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Oxytetracycline Hydrochloride



$C_{22}H_{24}N_2O_9 \cdot HCl$: 496.89

(4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(dimethylamino)-3,5,6,10,11,12a-hexahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,5a,6,12,12a-octahydrotetracene-2-carboxamide hydrochloride [*2058-46-0*]

Oxytetracycline Hydrochloride is the hydrochloride of a tetracycline substance having antibacterial activity produced by the growth of *Steptomyces rimosus*.
Oxytetracycline Hydrochloride contains not less than 880 μg (potency) and not more than 945 μg (potency) per mg of oxytetracycline ($C_{22}H_{24}N_2O_9$: 460.43), calculated on the dried basis.

Description Oxytetracycline Hydrochloride appears as yellow crystals or crystalline powder.
Oxytetracycline Hydrochloride is freely soluble in water, and practically insoluble in ethanol (99.5).

Identification (1) Determine the absorption spectra of solutions of Oxytetracycline Hydrochloride and Oxytetracycline Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Weigh 20 mg of Oxytetracycline Hydrochloride, and dissolve in 3 mL of water, add one drop of silver nitrate TS: the solution turns to be turbid.

Specific Optical Rotation $[\alpha]_D^{20}$: -188 ~ -200° (0.25 g calculated on the dried basis, 0.1 mol/L hydrochloric acid, 25 mL, 100 mm).

pH The pH of a suspension obtained by suspending 0.1 g of Oxytetracycline Hydrochloride in 10 mL of water is between 2.0 and 3.0.

Absorbance Ratio Weigh accurately about 50 mg each of Oxytetracycline Hydrochloride and Oxytetracycline RS, dissolve each in 0.1 mol/L hydrochloric acid TS to make exactly 250 mL. Pipet 10 mL each of these solutions, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use these solutions as the test solution and the standard solution, respectively. Determine the absorbances at 353 nm, A_T and A_S, of the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry (92.5 ± 4.3 %).

$$\text{Absorbance ratio [\%]} = \frac{A_T}{A_S} \times \frac{\text{Amount [}\mu\text{g (potency)] of Oxytetracycline RS}}{\text{Amount [mg] of Oxytetracycline Hydrochloride}} \times \frac{100}{100 - m}$$

m: Water content (%) of Oxytetracycline Hydrochloride

Purity (1) ***Heavy metals***—Proceed with 0.5 g of Oxytetracycline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of standard lead solution (not more than 50 ppm).

(2) ***Related substances***—Weigh accurately about 20 mg of Oxytetracycline Hydrochloride, dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the test solution. Separately, weigh accurately about 20 mg of 4-epioxytetracycline, dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the 4-epioxytetracycline stock solution. Dissolve about 20 mg of tetracycline hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the tetracycline hydrochloride stock solution. Weigh accurately about 8 mg of *β*-apooxytetracycline, dissolve in exactly 5 mL of 0.01 mol/L sodium hydroxide TS, add 0.01 mol/L hydrochloric acid to make exactly 100 mL, and use this solution as the *β*-apooxytetracycline stock solution. Pipet 1 mL of the 4-epioxytetracycline stock solution, 4 mL of the tetracycline hydrochloride stock solution, and 40 mL of the *β*-apooxytetracycline stock solution, add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatograpy according to the following conditions, and determine each peak area of each solution by the automatic integration method: the peak areas of 4-epioxytetracycline and tetracycline from the test solution are not larger than the area of each respective peak from the standard solution, and the total area of the

peaks of α-apooxytetracycline, *β*-apooxy-tetracycline, and the peaks between α-apooxytetracycline and *β*-apooxytetracycline is not larger than the peak area of *β*-apooxytetracycline from the standard solution. The area of the peak of 2-acetyl-2-decarboxamide oxytetracycline, which appears after the principal peak from the standard solution, is not larger than 4 times the peak area of 4-epioxytetracycline from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (8 μm in particle diameter).

Column temperature: A constant temperature of about 60 °C

Mobile phase: Control the step or concentration gradient by mixing mobile phases A and B as directed in the following table.

Mobile phase A: Mix 60 mL of 0.33 mol/L potassium dihydrogen phosphate TS, 100 mL of a solution of tetrabutylammonium hydrogen sulfate (1 in 100), 10 mL of a solution of disodium dihydrogen ethylenediamine tetraacetate dihydrate (1 in 2500), and 200 mL of water, and adjust the pH to 7.5 with 2 mol/L sodium hydroxide TS. Add 30 g of *t*-butyl alcohol and water to make 1000 mL.

Mobile phase B: Mix 60 mL of 0.33 mol/L potassium dihydrogen phosphate TS, 50 mL of a solution of tetrabutylammonium hydrogen sulfate (1 in 100), 10 mL of a solution of disodium dihydrogen ethylenediamine tetraacetate dihydrate (1 in 2500), and 200 mL of water, and adjust the pH to 7.5 with 2 mol/L sodium hydroxide TS. Add 100 g of *t*-butyl alcohol and water to make 1000 mL.

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-20	70→10	30→90
20-35	10→20	90→80

Flow rate: 1.0 mL/minute

System suitability

Test for required detectability: Pipet 1 mL of the 4-epioxytetracycline stock solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL. Pipet 4 mL of this solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 20 mL. Confirm that the peak area of 4-epioxytetracycline obtained from 20 μL of this solution is equivalent to 14 to 26 % of the peak area of 4-epioxytetracycline from the standard solution.

System performance: Weigh accurately about 8 mg of *α*-apooxytetracycline, dissolve in exactly 5 mL of 0.01 mol/L sodium hydroxide TS, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the *α*-apooxytetracycline stock solution. Pipet 3 mL of the test solution, 2 mL of the 4-epioxytetracycline stock solution, 6 mL of the tetracycline hydrochloride stock solution, 6 mL of the *β*-apooxyteetracycline stock solution, and 6 mL of the *α*-apooxytetracycline stock solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, 4-epioxy-tetracycline, oxytetracycline, tetracycline, *α*-apooxy-tetracycline, and *β*-apooxyteetracycline are eluted in this order with the resolutions between the peaks of 4-epioxytetracycline and oxytetracycline, between oxytetracycline and tetracycline, and between *α*-apooxy-tetracycline and *β*-apooxyteetracycline being not less than 4, not less than 5, and not less than 4, respectively, and the symmetry factor of the peak of oxytetracycline being not more than 1.3.

System repeatability: Pipet 1 mL of the 4-epioxytetracycline stock solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL. When the test is repeated 6 times with 20 μL each of this solution under the above operating conditions, the relative standard deviation of the peak areas of 4-epioxytetracycline is not more than 2.0 %.

Relative retention time: The relative retention time of *α*-apooxytetracycline with respect to oxytetracycline is about 2.1.

Time span of measurement: About 3.5 times as long as the retention time of oxytetracycline beginning after the solvent peak

0.33 mol/L Potassium dihydrogen phosphate TS—Dissolve 4.491 g of potassium dihydrogen phosphate in water to make 100 mL.

Loss on Drying Not more than 2.0 % (1 g, in vacuum, 60 °C, 3 hours).

Residue on Ignition Not more than 0.5 % (1 g).

Sterility Test It meets the requirement, when Oxytetracycline Hydrochloride is used in a sterile preparation.

Bacterial Endotoxins Less than 0.4 EU/mg (potency) of oxytetracycline, when Oxytetracycline Hydrochloride is used in a sterile preparation.

Histamine It meets the requirement, when Oxytetracycline Hydrochloride is used in a sterile preparation. Weigh an appropriate amount of Oxytetracycline Hydrochloride, dissolve in water to make the solution so that each mL contains 5.0 mg (potency), and use the solution as the test solution. Use 0.6 mL of this solution for the test.

Assay Weigh accurately about 50 mg (potency) of Oxytetracycline Hydrochloride, dissolve in 25 mL of 0.1 mol/L hydrochloric acid TS, add water to make exactly 250 mL, and use this solution as the test solution. Separately, weigh accurately about 50 mg (poten-

cy) of Oxytetracycline RS, dissolve in 25 mL of 0.1 mol/L hydrochloric acid TS, add water to make exactly 250 mL, and use this solution as the standard solution. Keep the standard solution at not exceeding 5 °C and use within 7 days. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of oxyte-tracycline in each solution.

Amount [μg (potency)] of oxytetracycline ($C_{22}H_{24}N_2O_9$)
= Amount [μg (potency)] of Oxytetracycline RS

$$\times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Dissolve 50 g of *t*-butyl alcohol in water to make 200 mL, and add 60 mL of pH 7.5 phosphate buffer solution, 50 mL of *t*-butylammonium hydrogen sulfate, 10 mL of edetic acid TS, and water to make 1000 mL.

Flow rate: 1.4 mL/minute

System suitability

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of erythromycin is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Oxytetracycline Hydrochloride Capsules

Oxytetracycline Hydrochloride Capsules contain not less than 90.0 % and not more than 120.0 % of the labeled amount of oxytetracycline ($C_{22}H_{24}N_2O_9$: 460.44).

Method of Preparation Prepare as directed under Capsules, with Oxytetracycline Hydrochloride.

Identification Dissolve an amount of the powdered contents of Oxytetracycline Hydrochloride Capsules, equivalent to about 10 mg of oxytetracycline hydrochloride, in 20 mL of water. To 1 mL of this solution add 1 mL of a solution of sodium carbonate (1 in 100) and 1 mL of diazobenzene sulfonate TS: an orange-red color is produced.

Loss on Drying Not more than 5.0 % (0.1 g, 0.7 kPa, 60 °C, 3 hours).

Dissolution Test Perform the test with 1 capsule of Oxytetracycline Hydrochloride Capsules at 75 revolutions per minute according to Method 2 under Dissolultion Test, using 900 mL of water as the dissolution solution. Take the dissolved solution 60 minutes after the start of the test, filter, discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL, and use this solution as the test solution. Separately, weigh accurately a suitable amount of Oxytetracycline RS, dissolve in 5 mL of 0.1 mol/L hydrochloric acid, add the dissolution solution to make the same concentration as the test solution, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 273 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry, using the dissolution solution as the blank. The dissolution rate of Oxytetracyline Hydrochloride Capsules in 60 minutes is not less than 80 % (Q).

Dissolution rate (%) with respect to the labeled amount of oxytetracycline ($C_{22}H_{24}N_2O_9$)

$$= C_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 90000$$

C_S: Concentration [mg (potency)/mL] of the standard solution

C: Labeled amount [mg (potency)] of oxytetracycline ($C_{22}H_{24}N_2O_9$) in 1 capsule

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 20 Oxytetracycline Hydrochloride Capsules. Weigh accurately a portion of the contents, equivalent to about 50 mg (potency) according to the labeled potency, dissolve in 25 mL of 0.1 mol/L hydrochloric acid TS, add water to make exactly 250 mL, and use this solution as the test solution. Separately, weigh accurately about 50 mg (potency) of Oxytetracycline RS, dissolve in 25 mL of 0.1 mol/L hydrochloric acid TS, add water to make exactly 250 mL, and use this solution as the standard solution. Keep the standard solution at a temperature not exceeding 5 °C, and use within 7 days. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of oxytetracycline hydrochloride in the test solution and standard solution.

Amount [μg (potency)] of oxytetracycline ($C_{22}H_{24}N_2O_9$)
= Amount [μg (potency)] of Oxytetracycline RS $\times \frac{A_T}{A_S}$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Dissolve 50 g of *t*-butyl alcohol in water to make 200 mL, and add 60 mL of pH 7.5 phosphate buffer solution, 50 mL of *t*-butylammonium hydrogen sulfate, 10 mL of edetic acid TS, and water to make 1000 mL.

Flow rate: 1.4 mL/minute

Containers and Storage ***Containers***—Tight containers.

Oxytetracycline Hydrochloride·Polymyxin B Sulfate Ophthalmic Ointment

Oxytetracycline Hydrochloride·Polymyxin B Sulfate Ophthalmic Ointment contains not less than 90.0 % and not more than 120.0 % of the labeled amount of oxytetracycline ($C_{22}H_{24}N_2O_9$: 460.44) and polymyxin B.

Method of Preparation Prepare as directed under Ophthalmic Ointments, with Oxytetracycline Hydrochloride and Polymyxin B Sulfate.

Identification (1) ***Oxytetracycline***—To an amount of Oxytetracycline Hydrochloride·Polymyxin B Sulfate Ophthalmic Ointment, equivalent to 25 mg (potency) of oxytetracycline hydrochloride according to the labeled potency, add 2.0 mL of methanol, warm for 20 minutes, filter, evaporate the filtrate to dryness in a water bath, and dissolve the residue in 20 mL of water. To 1 mL of this solution add 1 mL of a solution of sodium carbonate (1 in 100) and 1 mL of diazobenzene sulfonate TS: an orange-red color is produced.

(2) ***Polymyxin B***—(i) Perform the test with the solution from the Assay (2) as directed in the Identification (1) under Polymyxin B Sulfate.

(ii) To the solution from the Assay (2) add 0.5 mL of a solution of ninhydrin (1 in 1000) and 2 drops of pyridine, and boil for 1 minute: a blue color is produced.

Water Not more than 1.0 % (1.0 g, volumetric titration, direct titration).

Sterility Test It meets the requirement.

Test for Metal Particles It meets the requirement.

Assay (1) ***Oxytetracycline hydrochloride***—Liquid chromatography: Weigh accurately an amount of Oxytetracycline Hydrochloride·Polymyxin B Sulfate Ophthalmic Ointment, equivalent to about 20 mg (potency) of oxytetracycline according to the labeled potency, transfer to a separatory funnel, add 50 mL of ether, shake vigorously, extract with three 25 mL volumes of 0.1 mol/L hydrochloric acid TS, combine the extracts, and add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Filter if necessary. Pipet a suitable volume of this solution, dilute with 0.1 mol/L hydrochloric acid TS, and use this solution as the test solution. Separately, weigh accurately about 50 mg (potency) of Oxytetracycline RS, dissolve in 25 mL of 0.1 mol/L hydrochloric acid TS, add water to make exactly 250 mL, and use this solution as the standard solution. Keep the standard solution at a temperature not exceeding 5 °C, and use within 7 days. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of oxytetracycline hydrochloride in each solution.

Amount [μg (potency)] of oxytetracycline ($C_{22}H_{24}N_2O_9$)
= Amount [μg (potency)] of Oxytetracycline RS $\times \frac{A_T}{A_S}$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

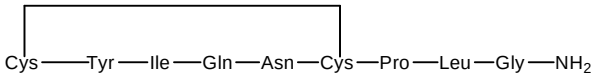
Mobile phase: Dissolve 50 g of *t*-butyl alcohol in water to make 200 mL, and add 60 mL of pH 7.5 phosphate buffer solution, 50 mL of *t*-butylammonium hydrogen sulfate, 10 mL of edetic acid TS, and water to make 1000 mL.

Flow rate: 1.4 mL/minute

(2) ***Polymyxin B sulfate***—The Cylinder-plate method: Proceed as directed in the Assay under Polymyxin B Sulfate. Prepare the test solution as follows. Weigh accurately an amount of Oxytetracycline Hydrochloride·Polymyxin B Sulfate Ophthalmic Ointment, equivalent to about 50000 units (potency) of polymyxin B sulfate according to the labeled potency, transfer to a 50 mL stoppered centrifuge tube, add 20 mL of petroleum ether, shake until homogeneous, centrifuge, and discard the clear supernatant liquid. Repeat the same procedure 1 to 2 times. To the residue add 1 % phosphate buffer solution (pH 6.0), shake, and make exactly 10 mL. Pipet a suitable volume of this solution, dilute with 1 % phosphate buffer solution (pH 6.0) to make the concentration of (3), and use this solution as the test solution.

Containers and Storage ***Containers***—Tight containers.

Oxytocin



$C_{43}H_{66}N_{12}O_{12}S_2$: 1007.19

1-({(4*R*,7*S*,10*S*,13*S*,16*S*,19*R*)-19-amino-7-(2-amino-2-oxoethyl)-10-(3-amino-3-oxopropyl)-16-(4-hydroxybenzoyl)-13-[(1*S*)-1-methylpropyl]-6,9,12,15,18-pentaoxo-1,2-dithia-5,8,11,14,17-pentaazacycloicosan-4-yl}carbonyl)-L-prolyl-L-leucylglycinamide [*50-56-6*]

Oxytocin is a synthetic peptide having the property of causing the contraction of uterine smooth muscle.
Oxytocin contains not less than 540 oxytocin Units and not more than 600 oxytocin Units per mg, calculated on the anhydrous and acetic acid-free basis.

Description Oxytocin appears as white powder.
Oxytocin is very soluble in water, and freely soluble in ethanol (99.5).
Oxytocin dissolves in hydrochloric acid TS.
The pH of the solution prepared by dissolving 0.10 g of Oxytocin in 10 mL of freshly boiled and cooled water is between 4.0 and 6.0.
Oxytocin is hygroscopic.

Identification Determine the absorption spectra of solutions of Oxytocin and Oxytocin RS, respectively, in water (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Constituent Amino Acids Weigh about 1 mg of Oxytocin, transfer into a test tube for hydrolysis, add 6 mol/L hydrochloric acid TS to dissolve, replace the air in the tube with Nitrogen, seal the tube under reduced pressure and heat at 110 to 115 ºC for 16 hours. After cooling, open the tube, evaporate the hydrolyzate to dryness under reduced pressure, add 2 mL of 0.02 mol/L hydrochloric acid TS to dissolve the residue, and use this solution as the test solution. Separately, weigh accurately about 24 mg of L-aspartic acid, about 24 mg of L-threonine, about 21 mg of L-serine, about 29 mg of L-glutamic acid, about 23 mg of L-proline, about 15 mg of glycine, about 18 mg of L-alanine, about 23 mg of L-valine, about 48 mg of L-cystine, about 30 mg of methionine, about 26 mg of L-isoleucine, about 26 mg of L-leucine, about 36 mg of L-tyrosine, about 33 mg of phenylalanine, about 36 mg of L-lysine hydrochloride, about 42 mg of L-histidine hydrochloride monohydrate and about 42 mg of L-arginine hydrochloride, dissolve them in 10 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the respective molar ratios with respect to leucine: 0.95 ~ 1.05 for aspartic acid, 0.95 ~ 1.05 for glutamic acid, 0.95 ~ 1.05 for proline, 0.95 ~ 1.05 for glycine, 0.80 ~ 1.10 for isoleucine, 0.80 ~ 1.05 for tyrosine and 0.80 ~ 1.05 for cystine, and not more than 0.01 each for others.

Operating conditions

Detector: A visible spectrophotometer (wavelength: 440 nm and 570 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 8 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography (sodium type) composed with a sulfonated polystyrene copolymer (3 μm in particle diameter).

Column temperature: A constant temperature of about 57 ºC.

Chemical reaction bath temperature: A constant temperature of about 130 ºC.

Color developing time: About 1 minute.

Mobile phase: Control the gradient by mixing the mobile phase A, B and C as directed in the following table.

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)	Mobile phase C (vol %)
0-9	100	0	0
9-25	0	100	0
25-61	0	100→0	100→0
61-80	0	0	100

Prepare mobile phases A, B and C according to the following table.

Mobile phase	A	B	C
Citric acid monohydrate	19.80 g	22.00 g	6.10 g
Trisodium citrate dihydrate	6.19 g	7.74 g	26.67 g
Sodium chloride	5.66 g	7.07 g	54.35 g
Ethanol (99.5)	260.0 mL	20.0 mL	—
Benzyl alcohol	—	—	5.0 mL
Thioglycol	5.0 mL	5.0 mL	—

Lauromacrogol Solution (1 in 4)	4.0 mL	4.0 mL	4.0 mL
Caprylic acid	0.1 mL	0.1 mL	0.1 mL
Water	a sufficient amount	a sufficient amount	a sufficient amount
Total amount	2000 mL	1000 mL	1000 mL
pH	3.3	3.2	4.9

Flow rate of mobile phase: About 0.26 mL/minute.

Flow rate of reaction reagent: About 0.3 mL/minute.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, cystine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine are eluted in this order with the resolutions between the peaks of threonine and serine, glycine and alanine, and isoleucine and leucine being not less than 1.5, 1.4 and 1.2, respectively.

System repeatability: When the test is repeated 3 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviations of the peak area of aspartic acid, proline, valine and arginine are not more than 2.0 %, respectively.

Reaction reagent—Mix 407 g of lithium acetate dihydrate, 245 mL of acetic acid (100) and 801 mL of 1-methoxy-2-propanol, add water to make 2 L, stir for more than 10 minutes while passing Nitrogen, and use this solution as Solution A. Separately, to 1957 mL of 1-methoxy-2-propanol add 77 g of ninhydrin and 0.134 g of sodium borohydride, stir for more than 30 minutes while passing nitrogen, and use this solution as Solution B. Mix Solution A and Solution B before use.

Purity (1) ***Acetic acid***—Weigh accurately about 15 mg of Oxytocin, dissolve in the internal standard solution to make exactly 10 mL, and use this solution as the test solution. Separately, weigh accurately about 1 g of acetic acid (100), add the internal standard solution to make exactly 100 mL. Pipet 2 mL of this solution, add the internal standard solution to make exactly 200 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of acetic acid to that of the internal standard: the amount of acetic acid is not less than 6.0 % and not more than 10.0 %.

$$\text{Amount (\%) of acetic acid } (C_2H_4O_2) = \frac{Q_T}{Q_S} \times \frac{W_S}{W_T} \times \frac{1}{10}$$

W_S: Amount (mg) of acetic acid (100)

W_T: Amount (mg) of Oxytocin

Internal standard solution—A solution of propionic acid in the mobile phase (1 in 10000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength : 210 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 ºC .

Mobile phase: To 0.7 mL of phosphoric acid add 900 mL of water, adjust the pH to 3.0 with 8 mol/L sodium hydroxide TS, and add water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol and mix.

Flow rate: Adjust the flow rate so that the retention time of acetic acid is about 3 minutes.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, acetic acid and propionic acid are eluted in this order with the resolution between these peaks being not less than 14.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of acetic acid to that of the internal standard is not more than 2.0 %.

(2) ***Related substances***—Dissolve 25 mg of Oxytocin in 100 mL of the mobile phase A, and use this solution as the test solution. Perform the test with 50 μL of the test solution as directed under Liquid Chromatography according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of each peak other than Oxytocin is not more than 1.5 %, and the total of them is not more than 5.0 %.

Operating conditions

Detector, column, column temperature, mobile phase, flowing of mobile phase, and flow rate: proceed as directed in the operating conditions in the Assay.

System suitability

Test for required detectability: Pipet 1 mL of the test solution, add the mobile phase A to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase A to make exactly 10 mL. Confirm that the peak area of oxytocin obtained from 50 μL of this solution is equivalent to 5 to 15 % of that from 50 μL of the solution for system suitability test.

System performance: Pipet an adequate amount of oxytocin and vasopressin, dissolve in the mobile

phase A, so that each mL contains about 0.1 mg each of them. When the procedure is run with 50 μL of this solution under the above operating conditions, vasopressin and oxytocin are eluted in this order with the resolution between these peaks being not less than 14, and the symmetry factor of the peak of oxytocin is not more than 1.5.

System repeatability: When the test is repeated 6 times with 50 μL each of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of oxytocin is not more than 2.0 %.

Time span of measurement: About 2.5 times as long as the retention time of oxytocin.

Water Not more than 5.0 % (50 mg, coulometric titration).

Assay Weigh accurately an amount of Oxytocin, equivalent to about 13000 Units, dissolve in the mobile phase A to make exactly 100 mL, and use this solution as the test solution. Separately, dissolve 1 vial of the Oxytocin RS in the mobile phase A to make a known concentration solution containing each mL contains about 130 Units, and use this solution as the standard solution. Perform the test with exactly 25 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S of oxytocin, respectively.

$$\text{Units per mg of Oxytocin, calculated on the anhydrous and acetic acid-free basis} = \frac{A_T}{A_S} \times \frac{W_S}{W_T} \times 100$$

W_S: Units per mL of the standard solution

W_T: Amount (mg) of Oxytocin, calculated on the anhydrous and acetic acid-free basis

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength 220 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 ºC .

Mobile phase : Program as follows with mobile phase A and mobile phase B in gradient or in isocratic.

Mobile phase A: Dissolve 15.6 g of sodium dihydrogen phosphate hydrate in 1000 mL of water.

Mobile B: A mixture of water and acetonitrile (1:1).

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-30	70→40	36→60
30-30.1	40→70	60→30
30.1-45	70	30

Flow rate: About 1.0 mL/minute.

System suitability

System performance: Weigh accurately a portion of Oxytocin and vasopressin, add mobile phase A to obtain a solution having a known concentration of about 0.1 mg per mL. When the procedure is run with 25 μL of this solution under the above operating conditions, vasopressin and oxytocin are eluted in this order with the resolution between these peaks being not less than 14, and the symmetry factor of the peak of oxytocin is not more than 1.5.

System repeatability: When the test is repeated 6 times with 25 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of oxytocin is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—At a temperature between 2 ºC and 8 ºC.

Oxytocin Injection

Oxytocin Injection is an aqueous solution for injection. Oxytocin Injection contains not less than 90.0 % and not more than 110.0 % of the labeled oxytocin Units.

Method of Preparation Prepare as directed under Injections, with oxytocin obtained from the posterior lobe of the pituitary or synthetic oxytocin.

Description Oxytocin Injection is a colorless, clear liquid.

pH 2.5 ~ 4.5.

Sterility Test It meets the requirement of the Membrane filtration method under the Sterility Test.

Bacterial Endotoxins Less than 10 EU/oxytocin Unit.

Foreign Insoluble Matter Test It meets requirement .

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay Measure exactly a portion of Oxytocin Injection according to the labeled Units, dilute with the diluent so that each mL contains about 1 Unit, and use this solution as the test solution. Separately, dissolve 1 bottle of Oxytocin RS in the mobile phase A to make exactly 20 mL. Pipet a suitable volume concentration solution so that each mL contain about 1 Unit, and use this solution as the standard solution. Perform the test

with exactly 100 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of oxytocin.

$$\text{Units per mL of Oxytocin Injection} = W_S \times \frac{A_T}{A_S} \times \frac{b}{a}$$

W_S: Units per mL of the standard solution
a: Volume (mL) of Oxytocin Injection
b: Total volume (mL) of the test solution prepared by diluting with the diluents

Diluent—Dissolve 5 g of chlorobutanol, 1.1 g of sodium acetate trihydrate, 5 g of acetic acid (100) and 6 mL of ethanol (95) in water to make 1000 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Control the gradient by mixing the mobile phase A and B as directed in the following table.

Mobile phase A: Dissolve 15.6 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water.

Mobile phase B: A mixture of water and acetonitrile (1:1).

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0–30	70→40	30→60
30–30.1	40→70	60→30
30.1-45	70	30

Flow rate of mobile phase: 1.0 mL/minute.

System suitability

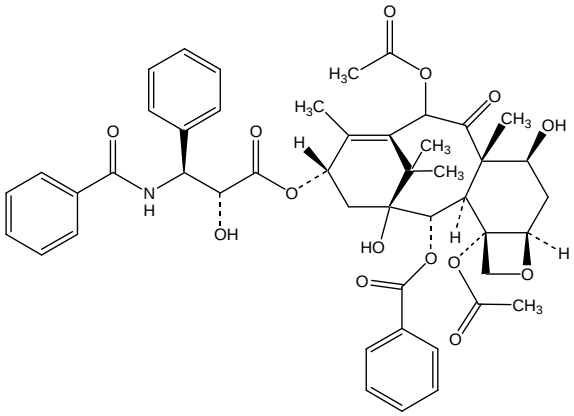
System performance: Measure exactly a portion each of oxytocin and vasopressin, dilute with mobile phase A so that each mL contains about 20 μg each. When the procedure is run with 100 μL of this solution under the above operating conditions, vasopressin and oxytocin are eluted in this order with the resolutions between these peaks being not less than 14, and the symmetry factor of the peak of oxytocin is not more than 1.5.

System repeatability: When the test is repeated 6 times with 100 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of oxytocin is not more than 2.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Storage—In a cold place, and avoid freezing.

Paclitaxel



$C_{47}H_{51}NO_{14}$: 853.91

(*1S*,2*S*,3*R*,4*S*,7*R*,9*S*,10*S*,12*R*,15*S*)-4,12-*bis*(acetyloxy)-1,9-dihydroxy-15-{[(2*R*,3*S*)-2-hydroxy-3-phenyl-3-(phenylformamido)propanoyl]oxy}-10,14,17,17-tetramethyl-11-oxo-6-oxatetracyclo[11.3.1.0^{3,10}.0^{4,7}]heptadec-13-en-2-yl benzoate [*33069-62-4*]

Paclitaxel contains not less than 97.0 % and not more than 102.0 % of paclitaxel ($C_{47}H_{51}NO_{14}$), calculated on the anyhydrous and solvent-free basis.

Description Paclitaxel appears as white powder.
Paclitaxel is soluble in ethanol (95) and practically insoluble in water.

Identification (1) Determine the infrared spectra of Paclitaxel and Paclitaxel RS, as directed in the potassium bromide disk method under lnfrared Spectrophotornetry : both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) The retention times of the principal peaks from the test solution and the standard solution in Assay are same.

Specific Optical Rotation $[\alpha]_D^{20}$: -49.0 ~ -55.0º (0.2 g, calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Paclitaxel according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—(i) When labeled as isolated from natural products, weigh about 10 g of Paclitaxel, add a mixture of methanol and acetic acid (100) (200 : 1), dissolve by sonication, if necessary, to make exactly 10 mL and use this as the test solution. Perform the test with 15 μL of the test solution as directed under Liquid Chromatography according to the following conditions. Measure the amount (%) of each

related substance: the amounts (%) of the related substances in Table 1 are not more than the amounts (%) specified in Table 1, the amount of each related substance is not more than 0.1 %, and the total amount of related substances is not more than 2.0 %.

$$\text{Amount (\%) of each related substance} = 100 \times \frac{FA_i}{A_u}$$

F : Relative response factor for each related substance peak (see Table 1)
A_i : Peak area of each related substance
A_u : Peak area of paclitaxel

Table 1

Relative retention time	Relative response factor (F)	Name	Limit (%)
0.24	1.29	Baccatin III	0.2
0.53	1.00	10-Deacetylpaclitaxel	0.5
0.57	1.00	7-Xylosylpaclitaxel	0.2
0.78	1.26	Cephalomannine (paclitaxel related substance I)	a_1[1]
0.78	1.26	2",3"-Dihydrocephalomannine	a_2[1]
0.86	1.00	10-Deacetyl-7-epipaclitaxel (paclitaxel related substance II)	0.5
1.10	1.00	Benzyl analog[3]	b_1[2]
1.10	1.00	3",4"-Dehydropaclitaxel C	b_2[2]
1.40	1.00	7-Epicephanlomannine	0.3
1.85	1.00	7-Epipaclitaxel	0.5

[1] Resolution may be imcomplete for these peaks, depending upon the relative amounts present; the sum of a_1 and a_2 is not more than 0.5 %.
[2] Resolution may be imcomplete for these peaks depending upon the relative amounts present; the sum of b_1 and b_2 is not more than 0.5 %.
3 The following chemical name is assigned to the related substance, benzyl analog: Baccatin III 13-ester with (2*R*,3*S*)-2-hydroxy-3-phenyl-3-(3-phenylacetylamino) propanoic acid.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 227 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with pentafluorophenylpropyl silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30 °C.

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: Acetonitrile
Mobile phase B: Water

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-35	35	65
35-60	35→80	65→20
60-70	80→35	20→65
70-80	35	65

Flow rate: 2.6 mL/minute.

System suitability

System performance: Weigh 10 mg each of paclitaxel related substance I RS {cephalomannine} and paclitaxel related substance II RS {10-Deacetyl-7-epipaclitaxel}, and dissolve in methanol to make 100 mL. Pipet 5 mL of this solution, and add methanol to make 50 mL. Pipet 5.0 mL of this solution, and add a mixture of methanol and acetic acid (100) (200 : 1) to make 50 mL. When the procedure is run with 15 μL of this solution under the above operating conditions, the relative retention time of the paclitaxel related substance I peak and the paclitaxel related substance II peak to the paclitaxel peak is about 0.78 and 0.86, respectively, and the resolution between these peaks is not less than 1.0.

System repeatability: Weigh about 10 mg of Paclitaxel RS, and dissolve in a mixture of methanol and acetic acid (100) (200 : 1), with sonication, if necessary, to make 100 mL. When the test is repeated 5 times with 15 μL each of this solution under the above operating conditions, the relative standard deviation of the peak area of paclitaxel is not more than 2.0 %.

(ii) When labeled as produced by a semisynthetic process, weigh about 10 mg of Paclitaxel, dissolve in a mixture of methanol and acetic acid (100) (200 : 1), with sonication, if necessary, to make exactly 10 mL and use this solution as the test solution. Perform the test with 15 μL each of the mixture of methanol and acetic acid (100) (200 : 1) and the test solution as directed under Liquid Chromatography according to the following conditions. Measure the amount (%) of each related substance excluding the peaks obtained from the mixture of methanol and acetic acid (100) (200 : 1): the amounts (%) of related substances in Table 2 are not more than the amounts (%) specified in Table 2, the amount of each related substance is not more than 0.1 %, and the total amount of related substances is not more than 2.0 %.

$$\text{Amount (\%) of each related substance} = 100 \times \frac{FA_i}{A_S}$$

F : Relative response factor for each related sub-

stance peak (see Table 2)

A_i : Peak area of each related substance

A_S : Sum of the areas of all the peaks obtained from the test solution

Table 2

Relative retention time	Relative response factor (*F*)	Name	Limit (%)
0.11	1.24	10-Deacetylbaccatin III	0.1
0.20	1.29	Baccatin III	0.2
0.42	1.39	Photodegradant[2]	0.1
0.47	1.00	10-Deacetylpaclitaxel	0.5
0.80	1.00	2-Debemzoylpaclitaxel-2-pentenoate	0.7
0.92[1]	1.00	Oxetane ring opened, acetyl and benzoyl[2]	x_1
0.92[1]	1.00	10-Acetoacetylpaclitaxel	x_2
0.94[1]	1.00	10-Deacetyl-7-epipaclitaxel (paclitaxel related substance II)	x_3
1.37	1.00	7-Epipaclitaxel	0.4
1.45	1.00	10,13-Bissidechainpaclitaxel[2]	0.5
1.54	1.00	7-Acetylpaclitaxel	0.6
1.80	1.75	13-Tes-baccatin III	0.1
2.14	1.00	7-Tes-paclitaxel	0.3

[1] Resolution may be imcomplete for these peaks, depending upon the relative amounts present; the sum of x_1, x_2 and x_3 is not more than 0.4 %.

[2] The following chemical names are assigned to the related substances Photodegradant, Oxetane ring opened, acetyl and benzoyl, and 10,13-Bussidechainpaclitaxel:

Photodegradant: (1*R*,2*R*,4*S*,5*S*,7,10*S*,11*R*,12*S*,13*S*,15*S*,16*S*)-2,10-diacetyloxy-5,13-dihydroxy-4,16,17,17-tetramethyl-8-oxa-3-oxo-12-phenylcarbonyloxypentacyclo [11.3.1.$0^{1.11}$.$0^{4.11}$.$0^{7.10}$]heptadec-15-yl (2*R*,3*S*)-2-hydroxy-3-phenyl-3-(phenylcarbonylamino)propanoate

Oxetane ring opened, acetyl and benzoyl migrated: (1*S*,2*S*,3*R*,4*S*,5*S*,7*S*,8*S*,10*R*,13*S*)-5,10-diacetyloxy-1,2,4,7-tetrahy droxy-8,12,15,15-tetramethyl-9-oxo-4-(phenylcarbonyloxymethyl) tricycle[9.3.1.$0^{3.8}$]pentadec-11-en-13-yl (2*R*,3*S*)-2-hydroxy-3-phenyl-3-(phenylcarbonylamino)pronoate

10,13-Bussidechainpaclitaxel: Baccatin III 13-ester with (2*R*,3*S*)-2-hydroxy-3-phenyl-3-(phenylcarbonylamino)propanoic acid, 10-ester with (2*S*,3*S*)-2-hydroxy-3-phenyl-3-(phenylcarbonylamino) propanoic acid

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 227 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 35 °C.

Mobile phase: Control the gradient by mixing the mobile phase A and B as directed in the following table.

Mobile phase A: A mixture of water and acetonitrile (3 : 2)

Mobile phase B: Acetonitrile

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-20	100	0
20-60	100→10	0→90
60-62	10→100	90→0
72-70	100	0

Flow rate: about 1.2 mL/minute.

System suitability

System performance: Weigh 96 mg of Paclitaxel RS, dissolve in a mixture of methanol and acetic acid (100) (200 : 1) by shaking to make 10 mL and use this solution as the standard solution (1). Separately, weigh 8 mg of paclitaxel related substance II RS {10-Deacetyl-7-epipaclitaxel}, dissolve in a mixture of methanol and acetic acid (100) (200 : 1) by shaking to make 100 mL and use this solution as the standard solution (2). Pipet 5 mL each of the standard solution (1) and the standard solution (2), add a mixture of methanol and acetic acid (100) (200 : 1) to make exactly 50 mL and use this solution as the system suitability solution. When the procedure is run with 15 μL of this solution under the above operating conditions, the relative retention time of the paclitaxel related substance II peak and the paclitaxel peak is about 0.94 and 1.0, respectively, and the resolution between these peaks is not less than 1.2.

System repeatability: When the test is repeated 5 times with 15 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the peak area of paclitaxel is not more than 2.0 %.

Water Not more than 4.0 % (0.1 g, coulometric titration)

Residue on Ignition Not more than 0.2 % (1 g).

Microbial Limit The total aerobic microbial count is not more than 100 CFU/g, the total combined yeasts/mould count is not more than 100 CFU/g, and *Escherichia coli*, *Salmonella* species, *Pseudomonas*

aeruginosa, and *Staphylococcus aureus* are not observed.

Bacterial Endotoxins Less than 0.4 EU/mg.

Assay Weigh acculately about 10 mg each of Paclitaxel and Paclitaxel RS, dissolve in a mixture of methanol and acetic acid (100) (200 : 1), with sonication, if necessary, to make exactly 10 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, measure the peak areas of paclitaxel obtained from each solutions, A_T and A_S.

$$\text{Amount (mg) of paclitaxel } (C_{47}H_{51}NO_{14}) = \text{Amount (mg) of Paclitaxel RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 227 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with pentafluorophenylpropyl silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of water and acetonitrile (11 : 9)

Flow rate: 1.5 mL/minute

System suitability

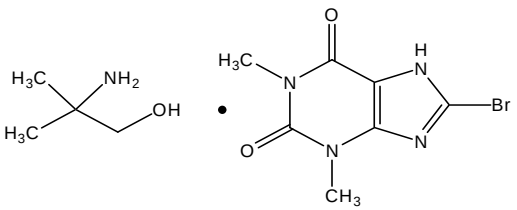
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the symmetry factor of the paclitaxel peak is between 0.7 and 1.3.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paclitaxel is not more than 1.5 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and at the temperature between 20 °C and 25 °C.

Pamabrom



$C_{11}H_{18}BrN_5O_3$: 348.20

2-Amino-2-methylpropan-1-ol; 8-bromo-1,3-dimethyl-7*H*-purine-2,6-dione [*606-04-2*]

Pamabrom contains not less than 72.2 % and not more than 76.6 % of 8-bromotheophylline ($C_7H_7BrN_4O_2$) and not less than 24.6 % and not more than 26.6 % of 2-amino-2-methyl-1-propanol ($C_4H_{11}NO$), calculated on the anhydrous basis.

Description Pamabrom appears as white powder. Pamabrom is sparingly soluble in water.

Identification Weigh 25 mg of Pamabrom, dissolve in 25 mL of water, by shaking to make exactly 100 mL and use this solution as the test solution. Separately, weigh 20 mg of 8-bromotheophylline RS, add 25 mL of water, 50 mL of methanol and a small amount of dilute ammonia TS, dissolve by gently shaking, add methanol to make 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solutions, as directed under Thin Layer Chromatography. Spot 10 μL each of the test solution and the standard solution on silica gel plate with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of xylene, methanol and acetic acid (100) (11 : 2 : 1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the R_f value of the principal spot from the test solution corresponds to that obtained from the standard solution.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Pamabrom according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Theophylline***—Weigh accurately about 0.2 g of Pamabrom, add 50 mL of a mixture of water and methanol (70 : 30), dissolve by sonication for 5 minutes, cool, add a mixture of water and methanol (70 : 30) to make exactly 200 mL and use this solution as the test solution. Separately, weigh accurately about 0.1 g of theophylline RS, dissolve in a mixture of water and methanol (70 : 30) by shaking, add 3 drops of ammonia solution (28) and a mixture of water and methanol (70 : 30) to make exactly 100 mL. Pipet 1.0 mL of this solution, add a mixture of water and methanol (70 : 30) to make exactly 200 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution according to the operating conditions of 8-Bromotheophylline under the Assay as directed under Liquid Chromatography, and measure the peak areas of theophylline obtained from each solutions, A_T and A_S.

$$\text{Amount (\%) of theophylline} = 20 \times \frac{C}{W} \times \frac{A_T}{A_S}$$

C: Concentration (μg/mL) of theophylline RS in the

standard solution
W: Amount (mg) of Pamabrom

Water Not more than 3.0 % (1.0 g, volumetric titration, direct titration)

Assay (1) ***8-Bromotheophylline***—Weigh accurately about 0.2 g of Pamabrom, add 50 mL of a mixture of water and methanol (70 : 30) amd 2 drops of ammonia solution (28), dissolve by sonication for 5 minutes, cool, add a mixture of water and methanol (70 : 30) to make exactly 200 mL. To 5.0 mL of this solution, add 10.0 mL of the internal standard solution and the mobile phase to make exactly 100 mL, filter, and use the filtrate as the test solution. Separately, weigh accurately about 75 mg of 8-Bromotheophylline RS, dissolve in a mixture of water and methanol (70 : 30) by shaking, add 2 drops of ammonia solution (28) and a mixture of water and methanol (70 : 30) to make exactly 100 mL. To 5.0 mL of this solution, add 10.0 mL of the internal standard and the mobile phase to make exactly 100 mL, filter, use the filtrate as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios of areas of 8-bromotheophylline peak, Q_T and Q_S, to the area of the internal standard peak, obtained from the test solution and the standard solution, respectively.

$$\text{Amount (mg) of 8-bromotheophylline } (C_7H_7BrN_4O_2) = 400 \times C \times \frac{Q_T}{Q_S}$$

C: Concentration (mg/mL) of 8-Bromotheophy lline RS in the standard solution

Internal standard solution—Weigh 12.5 mg of caffeine and dissolve in a mixture of water and methanol (70 : 30) to make exactly 100 mL.

Operating conditions
Detector: An ultraviolet absorption photometer (wavelength: 280 nm).
Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 to 10 μm in particle diameter).
Mobile phase: A mixture of water, methanol and acetic acid (100) (69 : 30 : 1)
System suitability
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the relative retention times of caffeine and 8-bromotheophylline are about 0.6 and 1.0, respectively and the resolution is not less than 2.0.
System repeatability: When the test is repeated 5 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of 8-bromotheophylline is not more than 2.0 %.

(2) ***2-Amino-2-methyl-1-propanol***—Weigh accurately about 1 g of Pamabrom, dissolve in 10 mL of water by warming gently on a steam bath until the solution is clear. Cool and and titrate with 0.5 mol/L hydrochloric acid VS (indicator: methyl orange TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.5 mol/L hydrochloric acid VS
= 44.57 mg of $C_4H_{11}NO$

Containers and Storage ***Containers***—Well-closed containers.

Pancreatin

[*8049-47-6*]

Pancreatin is a substance containing enzymes which have a starch digestive activity, fat digestive activity and protein digestive activity, obtained from the pancreas of the editable animal, mainly the hog. Pancreatin contains, in each gram, not less than 2800 units of starch saccharifying activity, not less than 28000 units of protein digestive activity and not less than 960 units of fat digestive activity. Pacreatin is usually diluted by appropriate excipients.

Description Pancreatin is a white to pale yellow powder and has a characteristic odor.

Purity (1) ***Deterioration***—Pancreatin has no unpleasant and deteriorated odor and taste.
(2) ***Fat***—Take 1 g of Pancreatin, add 20 mL of ether, mix with shaking occasionally, extract for 30 minutes, filter, wash the filtrate with 10 mL of ether, combine the filtrate and the washing, evaporate the ether and dry the residue at 105 ºC for 2 hours: the amount is not more than 20 mg.

Loss on Drying Not more than 4.0 % (1 g, in vacuum, P_2O_5, 24 hours).

Residue on Ignition Not more than 5.0 % (1 g).

Microbial Limit *Escherichia coli* and *Salmonella* species are not observed.

Assay (1) ***Starch digestive activity***—(i) Test solution: Weigh accurately about 0.1 g of Pancreatin and add a suitable amount of water, cooled with ice, to make exactly 100 mL by shaking well. Pipet 10 mL of this solution and add water, cooled with ice, to make exactly 100 mL.
(ii) Substrate solution: Use potato starch TS for measuring the starch digestive activity. But, add 10 mL

of phosphorus buffer solution for Pancreatin instead of 10 mL of 1 mol/L acetic acid-sodium acetate buffer solution, pH 5.0.

(iii) Operating method: Proceed according to the measurement of starch saccharification activity in the Assay for starch digestive activity in Digestion Test.

(2) ***Protein digestive activity***—(i) Test solution: Weigh accurately about 0.1 g of Pancreatin and add a suitable amount of water, cooled with ice, by shaking well and add water, cooled with ice, to make exactly 200 mL.

(ii) Substrate solution: Use substrate solution (2) for Assay for protein digestive activity in the Digestion Test. Adjust pH to 8.5.

(iii) Operating method: Proceed according to Assay for protein digestive activity in the Test for digestion (2). But, trichloroacetic acid B TS is used as a precipitating agent.

(3) ***Fat digestive activity***—(i) Test solution: Weigh accurately about 0.1 g of Pancreatin and add a suitable amount of water, cooled with ice, by shaking well and then add water, cooled with ice, to make exactly 100 mL.

(ii) Emulsion: Weigh 18 g of polyvinyl alcohol I and 2 g of polyvinyl alcohol II and prepare according to the Assay for fat digestive activity in the Digestion Test.

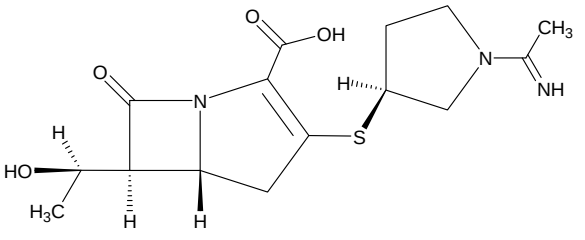
(iii) Substrate solution: Use the specified solution in Assay for fat digestive activity in the Digestion Test.

(iv) Operating method: Proceed according to Assay for fat digestive activity in the Digestion Test. But, phosphorus buffer solution, pH 8.0, is used as a buffer solution.

Containers and Storage ***Containers***—Tight containers.

Storage—Not exceeding 30 ºC.

Panipenem



$C_{15}H_{21}N_3O_4S$: 339.41

(5*R*,6*S*)-3-[(3*S*)-1-Ethanimidoylpyrrolidin-3-yl]sulfanyl-6-[(1*R*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid [*87726-17-8*]

Panipenem contains not less than 900 μg (potency) and not more than 1010 μg (potency) per mg of panipenem ($C_{15}H_{21}N_3O_4S$), calculated on the anhydrous and solvent-free basis.

Description Panipenem appears as white to pale yellow powder or masses.

Panipenem is very soluble in water, freely soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in ether.

Panipenem is hygroscopic.

Panipenem deliquesces in the presence of moisture.

Identification (1) Dissolve 0.02 g of Panipenem in 2 mL of water, add 1 mL of hydroxylammonium hydrochloride-ethanol TS, allow to stand for 3 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS, and shake: a red-brown color develops.

(2) Determine the absorption spectra of solutions of Panipenem and Panipenem RS in 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0) (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit maxima between 296 and 300 nm.

(3) Determine the infrared spectra of Panipenem and Panipenem RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit absorption at the wave numbers of about 1760 cm^{-1}, 1676 cm^{-1}, 1632 cm^{-1}, 1588 cm^{-1}, 1384 cm^{-1}, and 1249 cm^{-1}.

Absorbance $E_{1cm}^{1\%}$ (298 nm): 280 ~ 310 (50 mg calculated on the anhydrous and solvent-free basis, 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0), 2500 mL).

Specific Optical Rotation $[\alpha]_D^{20}$: +55 ~ +65° (0.1 g calculated on the anhydrous and solvent-free basis, 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0), 10 mL, 100 mm).

pH Dissolve 0.5 g of Panipenem in 10 mL of water: the pH of this solution is between 4.5 and 6.5.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Panipenem according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Residual solvents***—Weigh accurately about 0.2 g of Panipenem, transfer to a 20 mL narrow-mouthed cylindrical glass bottle, add exactly 2 mL of the internal standard solution and 2 mL of water to dissolve, seal tightly a rubber stopper with aluminum cap, and use this solution as the test solution. Separately, pipet 15 mL of ethanol (99.5) and 3 mL of acetone, and add water to make exactly 200 mL. Pipet 1 mL and 2 mL of this solution, and add water to each to make exactly 20 mL. Transfer exactly 2 mL each of these solutions to a 20 mL narrow-mouthed cylindrical glass bottle, add exactly 2 mL of the internal standard solution, seal tightly a rubber stopper with aluminum cap, and use

these solutions as the standard solution (1) and standard solution (2). Shake gently in a water bath at a constant room temperature, and allow to stand for 30 minutes. Perform the test with 1 mL of the gas in each container as directed under Gas chromatography according to the following conditions. Calculate the ratios, Q_{Ta} and Q_{Tb}, of the peak area of ethanol and acetone to that of the internal standard from the test solution, the ratios, Q_{Sa1} and Q_{Sb1}, of the peak area of ethanl and acetone to that of the internal standard from the standard solution (1), and the ratios, Q_{Sa2} and Q_{Sb2}, of the peak area of ethanol and acetone to that of the internal standard from the standard solution (2). Calculate the amounts of ethanol and acetone by the following equations: not more than 5.0 % and not more than 1.0 %, respectively.

$$\text{Amount (\%) of ethanol} = 15 \times 0.79 \times \frac{Q_{Ta} + Q_{Sa2} - 2Q_{Sa1}}{2(Q_{Sa2} - Q_{Sa1})} \times \frac{1}{1000} \times \frac{100}{W}$$

W: Amount (g) of Panipenem taken

$$\text{Amount (\%) of acetone} = 3 \times 0.79 \times \frac{Q_{Tb} + Q_{Sb2} - 2Q_{Sb1}}{2(Q_{Sb2} - Q_{Sb1})} \times \frac{1}{1000} \times \frac{100}{W}$$

W: Amount (g) of Panipenem taken
0.79: Density (g/mL) of ethanol (99.5) and acetone

Internal standard solution—A solution of 1-propanol (1 in 400)

Operating conditions

Detector: A hydrogen flame ionization detector

Column: A gas column about 1 mm in internal diameter and about 40 m in length, packed with porous polymer beads for gas chromatography.

Column temperature: A constant temperature of about 140 °C

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of 1-propanol is about 6 minutes.

System suitability

System performance: When the procedure is run with 1 mL of the gas from the standard solution (2) under the above operating conditions, ethanol, acetone, and the internal standard are eluted in this order with the resolution between the peaks of ethanol and acetone being not less than 4.

System repeatability: When the test is repeated 6 times with 1 mL each of the gas from the standard solution (2) under the above operating conditions, the relative standard deviation of the ratio of the peak area of ethanol to that of the internal standard is not more than 5.0 %.

(3) ***Related substances***—Dissolve 50 mg (potency) of Panipenem in 50 mL of water, and use this solution as the test solution. Perform the test with 10 μL of the test solution immediately after preparation as directed under Liquid Chromatography according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of each peak by the area percentage method: the amount of each peak other than panipenem is not more than 2.0 %, and the total area of the peaks other than panipenem is not more than 6.0 %.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized porous glass for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: A mixture of 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 8.0) and acetonitrile (50 : 1)

Mobile phase B: A mixture of 0.01 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 8.0) and acetonitrile (3 : 1)

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-15	100	0
15-50	100→0	0→100

Flow rate: Adjust the flow rate so that the retention time of panipenem is about 16 minutes.

System suitability

Detection sensitivity: Adjust the detection sensitivity so that the peak height of panipenem obtained from 10 μL of a solution of Panipenem and betamipron (1 in 100000) composes 10 to 20 % of the full scale.

System performance: When the procedure is run with 10 μL of a solution of Panipenem and betamipron (1 in 100000) under the above operating conditions, panipenem and betamipron are eluted in this order with the resolution between these peaks being not less than 3.0.

Time span of measurement: About 3 times as long as the retention time of panipenem, beginning after the solvent peak.

Column washing—Use a mixture of 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 8.0) and acetonitrile (3 : 1) to wash the column after the test.

Water Weigh accurately about 0.5 g of Panipenem, transfer to a 15 mL narrow-mouthed cylindrical glass bottle, add exactly 2 mL of the internal standard solution to dissolve, seal tightly a rubber stopper with aluminum cap, and use this solution as the test solution. Separately, weigh accurately 2 g of water, and add the internal standard solution to make exactly 100 mL. Pipet 5 mL and 10 mL of this solution, add the internal standard solution to make exactly 20 mL, and use these solutions as the standard solution (1) and standard solution (2). Perform the test with 1 μL each of the test solution, standard solution (1), and standard solution (2) as directed under Gas Chromatography according to the following conditions, and calculate the ratios, Q_T, Q_{S1}, and Q_{S2}, of the peak area of water to that of the internal standard. Calculate the amount of water by the following equation: not more than 5.0 %.

$$\text{Water (\%)} = \frac{W_S}{W_T} \times \frac{Q_T + 7Q_{S2} - 2Q_{S1}}{2(Q_{S2} - Q_{S1})} \times \frac{1}{100} \times 100$$

W_S: Amount (g) of water taken
W_T: Amount (g) of Panipenem taken

Internal standard solution—A solution of acetonitrile in methanol (1 in 100)

Operating conditions

Detector: A thermal conductivity detector

Column: A gas column about 3 mm in internal diameter and about 2 m in length, packed with porous ethylvinylbenzene-divinylbenzene copolymer for gas chromatography (150 to 180 μm in particle diameter).

Column temperature: A constant temperature of about 125 °C

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of acetonitrile is about 8 minutes.

System suitability

System performance: When the procedure is run with 1 μL of standard solution (2) under the above operating conditions, water, methanol, and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 1 μL each of standard solution (2) under the above operating conditions, the relative standard deviation of the peak area of water to that of the internal standard is not more than 5.0 %.

Sterility Test It meets the requirement, when Panipenem is used in a sterile preaparation. Use rinsing fluid containing 0.7 % polysorbate 80.

Bacterial Endotoxins Less than 0.15 EU/mg (potency) of panipenem, when Panipenem is used in a sterilre preparation.

Assay Weigh accurately about 0.1 g (potency) each of Panipenem and Panipenem RS, and dissolve separately in 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0) to make exactly 100 mL. To 5 mL each of these solutions add 5 mL of the internal standard solution and 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0) to make 20 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of the test solution and standard solution within 30 minutes after preparation as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of panipenem to that of the internal standard in each solution.

$$\text{Amount [μg (potency)] of panipenem } (C_{15}H_{21}N_3O_4S) = \text{Amount [μg (potency)] of Panipenem RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of sodium *p*-styrenesulfonate in 0.02 mol/L 3-(*N*-morpholino)propane-sulfonic acid buffer solution (pH 7.0) (1 in 1000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silicone polymer coated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: A mixture of 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 8.0) and acetonitrile (50 : 1)

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 12 minutes.

System suitability

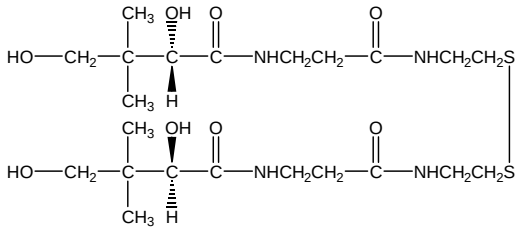
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, panipenem and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of panipenem to that of the internal standard is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Not exceeding -10 °C.

Pantethine



$C_{22}H_{42}N_4O_8S_2$: 554.72

N-[3-[2-[2-[3-[(2,4-Dihydroxy-3,3-dimethylbutanoyl)amino]propanoylamino]ethyldisulfanyl]ethylamino]-3-oxopropyl]-2,4-dihydroxy-3,3-dimethylbutanamide [*16816-67-4*]

Pantethine is an aqueous solution containing 80.0 % of pantethine.
Pantethine contains not less than 98.0 % and not more than 101.0 % of pantethine ($C_{22}H_{42}N_4O_8S_2$), calculated on the anhydrous basis.

Description Pantethine is a clear, colorless to pale yellow viscous liquid.
Pantethine is miscible with water, with methanol or with ethanol (95).
Pantethine is decomposed by light.

Identification (1) Take 0.7 g of Pantethine, add 5 mL of sodium hydroxide TS, shake and add 1 to 2 drops of copper (II) sulfate TS: a blue-purple color is observed.

(2) Take 0.7 g of Pantethine, add 3 mL of water, shake, add 0.1 g of zinc powder and 2 mL of acetic acid (100) and boil for 2 to 3 minutes. After cooling, add 1 to 2 drops of sodium nitroprusside TS: a red-purple color is observed.

(3) Take 1.0 g of Pantethine and add 500 mL of water and shake. To 5 mL of this solution, add 3 mL of 1 mol/L hydrochloric acid TS and heat in a water-bath for 30 minutes. After cooling, add 7 mL of a solution of hydroxylamine hydrochloride in sodium hydroxide TS (3 in 140) and allow to stand for 5 minutes. Add 3 drops of 2,4-dinitrophenol TS and add 1 mol/L hydrochloric acid TS drop-wise until the solution has no color and then add 1 mL of iron (III) chloride TS: a red-purple color is observed.

Specific Optical Rotation $[\alpha]_D^{20}$: +15.0 ~ +18.0º (1 g, calculated on the anhydrous basis, water, 25 mL, 100 mm).

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Pantethine according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Arsenic***—Prepare the test solution with 2.0 g of Pantethine according to Method 3 and perform the test (not more than 1 ppm).

(3) ***Related substances***—Dissolve 0.6 g of Pantethine in 10 mL of water and use this solution as the test solution. Pipet 2.0 mL of the test solution, add water to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 2 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with methyl ethyl ketone saturated with water to a distance of about 10 cm and air-dry the plate. Allow the plate to stand for about 10 minutes in iodide vapor: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

(4) ***Mercapto compounds***—To 1.5 g of Pantethine, add 20 mL of water, shake, add 1 drop of ammonia TS and 1 to 2 drops of sodium nitroprusside TS: a red color is not observed.

Water 18 ~ 22 % (0.2 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (2 g).

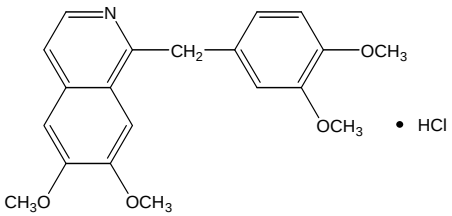
Assay Weigh accurately about 0.3 g of Pantethine, add water to make exactly 20 mL. Transfer exactly 5 mL of this solution in an iodine bottle and add exactly 25 mL of 0.05 mol/L bromine VS and 100 mL of water. Add 5 mL of diluted sulfuric acid (1 in 5) rapidly, stopper tightly immediately and warm at 40 ºC to 50 ºC for 15 minutes with occasional shaking. After cooling, carefully add 5 mL of a solution of potassium iodide (2 in 5), then immediately stopper tightly, shake, add 100 mL of water and titrate the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS). Perform a blank determination and make any necessary correction.

Each mL of 0.05 mol/L bromine VS
= 5.547 mg of $C_{22}H_{42}N_4O_8S_2$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and not exceeding 10 ºC.

Papaverine Hydrochloride



$C_{20}H_{21}NO_4$·HCl: 375.85

1-[(3,4-Dimethoxyphenyl)methyl]-6,7-dimethoxyisoquinoline hydrochloride [*61-25-6*]

Papaverine Hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of papaverine hydrochloride ($C_{20}H_{21}NO_4 \cdot HCl$).

Description Papaverine Hydrochloride appears as white crystals or crystalline powder.
Papaverine Hydrochloride is sparingly soluble in water or in acetic acid (100), slightly soluble in ethanol (95) and practically insoluble in acetic anhydride or in ether.

pH—The pH of a solution of Papaverine Hydrochloride (1 in 50) is between 3.0 and 4.0.

Identification (1) Take 1 mg of Papaverine Hydrochloride and add 1 drop of formalin-sulfuric acid TS: a colorless to a pale yellow-green color is observed and it gradually changes to deep red, then to brown.

(2) Dissolve 20 mg of Papaverine Hydrochloride in 1 mL of water and add 3 drops of sodium acetate TS: a white precipitate is produced.

(3) Dissolve 1 mg of Papaverine Hydrochloride in 3 mL of acetic anhydride and 5 drops of sulfuric acid, heat in a water-bath for 1 minute and examine under ultraviolet light (main wavelength: 365 nm): the solution shows a yellow-green fluorescence.

(4) Dissolve 0.1 g of Papaverine Hydrochloride in 10 mL of water, make alkaline with ammonia TS and shake with 10 mL of ether. Draw off the ether layer, wash with 5 mL of water and filter. Evaporate the filtrate on a water-bath and dry the residue at 105 °C for 3 hours: the residue so obtained melts between 145 °C and 148 °C.

(5) Alkalify a solution of Papaverine Hydrochloride (1 in 50) with ammonia TS and filter the precipitate. Acidify the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests (2) for chloride.

Purity (1) ***Clarity and color of solution***—Dissolve 0.10 g of Papaverine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) ***Related substances***—Weigh accurately 20 mg of Papaverine Hydrochloride, dissolve in the diluent to make exactly 10 mL, and use this solution as the test solution. Pipet 1.0 mL of the test solution, add the diluent to make exactly 100 mL, pipet 1.0 mL of this solution, add the diluent to make exactly 10 mL, and use this solution as the standard solution (1). Separately, weigh accurately 12 mg of Noscapine RS, dissolve in 1.0 mL of the test solution, add the diluent to make 100 mL, and use this solution as the standard solution (2). Perform the test with 10 μL each of the test solution and standard solutions (1) and (2) as directed under Liquid Chromatography according to the following conditions. Determine each peak area of each solution by the automatic integration method, and calculate the amount of related substances: papaverine related substance I {tetrahydropapaverine}, papaverine related substance II {dihydropapaverine}, papaverine related substance III {papaverinol}, noscapine, papaverine related substance IV {2-(3,4-dimethoxyphenyl)-*N*-[2-(3,4-dimethoxyphenyl)-ethyl]acetamide}, and papaverine related substance V {papaveraldine} are not more than 0.1 %, respectively. The peak area of any other related substance is not larger than the area of the principal peak obtained from the standard solution (1) (not more than 0.1 %), and the total peak area of related substances is not larger than 5 times the area of the principal peak from the standard solution (1) (not more than 0.5 %). Exclude any peak with an area less than 0.5 times the area of the principal peak from the standard solution (1) (not more than 0.05 %). Multiply the peak areas of papaverine related substance II, noscapine, and papaverine related substance V by their relative response factors, 2.7, 6.2, and 0.5, respectively.

Diluent—A mixture of mobile phase A and acetonitrile (80 : 20)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 238 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 25 cm in length, packed with octylsilyl silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A, B, and C as directed in the following table.

Mobile phase A: Dissolve 3.4 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 3.0 with dilute phosphoric acid.

Mobile phase B: Acetonitrile

Mobile phase C: Methanol

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)	Mobile phase C (vol %)
0-5	85	5	10
5-12	85→60	5	10→35
12-20	60	5	35
20-24	60→40	5→20	35→40
24-27	40	20	40
27-32	40→85	20→5	40→10
32-40	85	5	10

Flow rate: 1 mL/minute

System suitability

System performance: When the procedure is run with 10 μL of the standard solution (2) under the above operating conditions, the resolution between the peaks of noscapine and papaverine is not less than 1.5. The relative retention times of papaverine related substance I, papaverine related substance II, papaverine related substance III, noscapine, papaverine related substance IV, and papaverine related substance V with respect to

papaverine (retention time of about 23.4 minutes) are 0.7, 0.75, 0.8, 0.9, 1.1, and 1.2, respectively.

(3) ***Morphine***—Dissolve 10 mg of Papaverine Hydrochloride in 1 mL of water, add 5 mL of 1-nitroso-2-naphthol TS and 2 mL of a solution of potassium nitrate (1 in 10) and warm 40 °C. Add 1 mL of solution of sodium nitrate (1 in 5000) and warm at 40 °C. After cooling, shake the mixture with 10 mL of chloroform, centrifuge and separate the aqueous layer: the solution has no more color than a pale red color.

(4) ***Readily carbonizable substances***—Perform the test with 0.12 g of Papaverine Hydrochloride: the solution has no more color than Color Matching Fluid S or P.

Loss on Drying Not more than 1.0 % (1 g, 105 °C, 4 hours).

Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 0.5 g of Papaverine Hydrochloride, previously dried, dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) by warming, cool and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 37.585 mg of $C_{20}H_{21}NO_4 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Papaverine Hydrochloride Injection

Papaverine Hydrochloride Injection is an aqueous solution for injection. Papaverine Hydrochloride Injection contains not less than 95.0 % and not more than 105.0 % of the labeled amount of papaverine hydrochloride ($C_{20}H_{21}NO_4 \cdot HCl$: 375.85).

Method of Preparation Prepare as directed under Injections, with Papaverine Hydrochloride.

Description Papaverine Hydrochloride Injection is a clear, colorless liquid.

pH—3.0 ~ 5.0.

Identification (1) Take 1 mL of Papaverine Hydrochloride Injection and add 3 drops of sodium acetate TS: a white precipitate is produced.

(2) Dilute a volume of Papaverine Hydrochloride Injection, equivalent to 0.1 g of Papaverine Hydrochloride according to the labeled amount, with water to make 10 mL, render the solution alkaline with ammonia TS and shake with 10 mL of ether. Draw off the ether layer, wash with 5 mL of water and filter. Evaporate the filtrate in a water-bath to dryness and dry the residue at 105 °C for 3 hours: the residue so obtained melts between 145 °C and 148 °C.

(3) Proceed with 1 mg each of the residue obtained in (2) as directed in the Identification (1) and (3) under Papaverine Hydrochloride.

(4) Alkalify 2 mL of Papaverine Hydrochloride Injection with ammonia TS, filter the precipitate off and acidify the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests (2) for chloride.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 6.0 EU/mg of papaverine hydrochloride.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

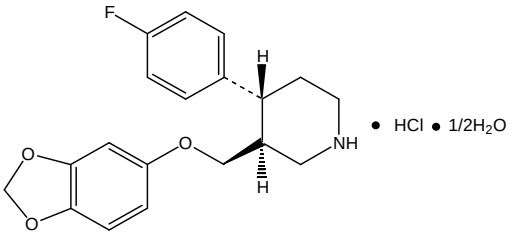
Assay Take exactly a volume of Papaverine Hydrochloride Injection, equivalent to about 0.2 g of papaverine hydrochloride ($C_{20}H_{21}NO_4.HCl$), add water to make 10 mL, render the solution alkaline with ammonia TS and extract with 20 mL, 15 mL, 10 mL and 10 mL of chloroform. Combine the extracts, wash with 10 mL of water and re-extract the washings with two 5 mL volumes of chloroform. Combine all the chloroform extracts and evaporate the chloroform in a water-bath. Dissolve the residue in 30 mL of acetic acid (100) and titrate with 0.05 mol/L perchloric acid VS (indicator: 2 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 18.793 mg of $C_{20}H_{21}NO_4 \cdot HCl$

Containers and Storage ***Containers***—Hermetic containers.

Storage—Light-resistant.

Paroxetine Hydrochloride Hydrate



$C_{19}H_{21}ClFNO_3 \cdot ½H_2O$: 374.84

(3*S*,4*R*)-3-[(2*H*-1,3-benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl)piperidine [*110429-35-1*]

Paroxetine Hydrochloride Hydrate contains not less than 97.5 % and not more than 102.0 % of paroxetine hydrochloride ($C_{19}H_{21}ClFNO_3$), calculated on the anhydrous basis.

Description Paroxetine Hydrochloride Hydrate appears as white, crystalline powder.
Paroxetine Hydrochloride Hydrate is freely soluble in methanol, sparingly soluble in ethanol (95) or in dichloromethane, and slightly insoluble in water.

Identification (1) To about 0.16 g of Paroxetine Hydrochloride Hydrate in a test tube, add 0.2 g of potassium dichromate and 1 mL of sulfuric acid and place a filter paper wet with 1,5-diphenylcarbohydrazide TS: the color of filter paper turns to red-purple.

(2) Determine the infrared spectra of Paroxetine Hydrochloride Hydrate and Paroxetine Hydrochloride Hydrate RS, as directed in the potassium bromide disk method under lnfrared Spectrophotornetry : both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears in the absorption spectra, dissolve Paroxetine Hydrochloride Hydrate and paroxetine hydrochloride hydrate RS in a mixture of 2-propanol and water (9 : 1) at the concentration of 10 % and recrystalize, respectively. Repeat the test on the residues.

(3) Perform the test with the test solution and the standard solution (3) in the Related substance I as directed under Liquid Chromatography, according to the operating conditions: the retention times of the principal peaks of the test solution and the standard solution are same.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Paroxetine Hydrochloride Hydrate in a platinum crucible according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) ***Related substance I***—Dissolve 0.1 g of Paroxetine Hydrochloride Hydrate, accurately weighed, in 20 mL of methanol, add the mobile phase to make exactly 100 mL and use this solution as the test solution. Pipet 1.0 mL of the test solution, add the mobile phase to make exactly 100 mL, pipet 1.0 mL of this solution, add the mobile phase to make exactly 10 mL and use this solution as the standard solution (1). Separately, dissolve 5 mg each of paroxetine related substance I {(3*S*,4*R*)-3[1,3-benzodioxol-5-yloxy]methyl}-4-(4-ethoxyphenyl)
piperidine ((+)-trans-paroxtine)} and Paroxetine Hydrochloride Hydrate RS in 2 mL of methanol, add the mobile phase to make exactly 10 mL and use this solution as the standard solution (2). Separately, dissolve 10 mg of Paroxetine Hydrochloride Hydrate RS in 2 mL of methanol, add the mobile phase to make exactly 10 mL and use this solution as the standard solution (3). Perform the test with 10 μL each of the test solution, the standard solution (1) and the standard solution (2) as directed under Liquid Chromatography, according to the following operating conditions and measure the areas of peaks: the area of peak corresponding to the related substance I obtained from the test solution is not more than 2 times the area of the principal peak from the standard solution (1). (not more than 0.2 %)

Operating conditions

Detector: An ultraviolet absorption photometer (main wavelength: 295 nm)

Column: A stainless steel column, about 4.0 mm in internal diameter and about 10 cm in length, packed with silica gel AGP for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of 0.58 % sodium chloride solution and methanol (8 : 2).

Flow rate: About 0.5 mL/minute

System suitability

System performance: When the procedure is run with 10 μL of the standard solution (2) according to the above operating conditions, the resolution between the related substance I and paroxetine is not less than 2.2.

Time span of measurement: About 2.5 times as long as the retention time of paroxetine.

(3) ***Related substance II***—Dissolve 50.0 mg of Paroxetine Hydrochloride Hydrate in a mixture of water and tetrahydrofuran (9 : 1) to make exactly 50 mL and use this solution as the test solution. To 5.0 mL of the test solution, add a mixture of water and tetrahydrofuran (9 : 1) to make exactly 50 mL and use this solution as the standard solution (1). Separately, dissolve 2 mg of paroxetine related substance II RS {(3*S*,4*R*)-3-[(1,3-benzodioxol-5-yloxy)methyl]-4-(4-ethoxyphenyl) piperidine} in a mixture of water and tetrahydrofuran (9 : 1) to make exactly 20 mL and use this solution as the standard solution (2). To 2.0 mL of the standard solution (1), add 2.0 mL of the standard solution (2) and a mixture of water and tetrahydrofuran (9 : 1) to make exactly 20 mL and use this solution as the standard solution (3). To 2.0 mL of the standard solution (1), add a mixture of water and tetrahydrofuran (9 : 1) to make exactly 200 mL and use this solution as the standard solution (4). Separately,

dissolve 2 mg of paroxetine related substance III RS {(3S,4R)-3-[(1,3-benzodioxol-5-yloxy)methyl]-4-phenypiperidien (desfluoroparoxetine)} in a mixture of water and tetrahydrofuran (9 : 1) to make exactly 20 mL and use this solution as the standard solution (5). Perform the test with 10 μL each of the test solution, the standard solution (3), the standard solution (4) and the standard solution (5) as directed under Liquid Chromatography, according to the following operating conditions and measure the areas of peaks: the area of the peak corresponding to the related substance III from the test solution is not more than 3 times the area of the principal peak from the standard solution (4) (0.3 %), area of each of any other related substances is not more than the area of the principal peak from the standard solution (4) (0.1 %), and the total area of those peaks is not more than 5 times the area of the principal peak from the standard solution (4) (0.5 %). The peaks with areas of less than 1/2 times the area of the principal peak from the standard solution (4) are disregarded.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 295 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter)

Column temperature: A constant temperature of about 40 °C.

Control the gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: A mixture of water, tetrahydrofuran and trifluoroactic acid (900 : 100 : 5)

Mobile phase B: A mixture of acetonitrile, tetrahydrofuran and trifluoroactic acid (900 : 100 : 5)

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-30	80	20
30-50	80→20	20→80
50-60	20	80
60-65	20→80	80→20
65-70	80	20

Flow rate: 1.0 mL/minute.

System suitability

System performance: Mix 2.0 mL of the standard solution (1) and 1.0 mL of the standard soluteon (5), and add a mixture of water and tetrahydrofuran (9 : 1) to make exactly 20 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, the relative retention time of the related substance III peak to the paroxetine peak is about 0.8. When the procedure is run with 20 μL of the standard solution (3) under the above operating conditions, the resolution between the peaks of the related substance II and paroxetine is not less than 3.5.

Time span of measurement: About 2.5 times as long as the retention time of paroxetine.

Water 2.2 ~ 2.7 % (0.3 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Dissolve about 50 mg each of Paroxetine Hydrochloride Hydrate and Paroxetine Hydrochloride Hydrate RS in water to make exactly 100 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography, according to the following operating conditions, and measure the peak areas, A_T and A_S, of paroxetine for the test solution and the standard solution, respectivly.

Amount (mg) of paroxetine hydrochloride ($C_{19}H_{21}ClFNO_3$) = Amount (mg) of paroxetine hydrochloride in Paroxetine Hydrochloride Hydrate RS, calcularated on the anhydrous basis $\times \frac{A_T}{A_S}$

Operating conditions

Detector: An ultraviolet absorption photometer (main wavelength: 295 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with trimethylsilyl silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Dissolve 3.85 g of ammonium acetate in 500 mL of water, adjust to pH 5.5 with acetic acid (100), add water to make 600 mL, add 400 mL of acetonitrile slowly and 10 mL of triethylamine and adjust to pH 5.5 with acetic acid (100).

Flow rate: 1 mL/minute.

System suitability

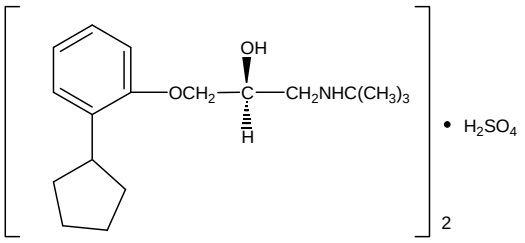
System performnace: Dissolve 5.0 mg each of Paroxetine Hydrochloride Hydrate and paroxetine related substance III RS, accurately weighed, in water to make exactly 10 mL. When the procedure is run with 10 μL of this solution according to the above operating conditions, the resolution between the peaks of paroxetine related substance III and paroxetine is not less than 2.0.

Time span of measurement: About 2 times as long as the retention time of paroxetine.

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Penbutolol Sulfate



$(C_{18}H_{29}NO_2)_2 \cdot H_2SO_4$: 680.94

(2*S*)-1-(*tert*-Butylamino)-3-(2-cyclopentylphenoxy) propan-2-ol;sulfuric acid [*38363-32-5*]

Penbutolol Sulfate, when dried, contains not less than 98.5 % and not more than 101.0 % of penbutolol sulfate [$(C_{18}H_{29}NO_2)_2 \cdot H_2SO_4$].

Description Penbutolol Sulfate is a white crystalline powder.
Penbutolol Sulfate is very soluble in acetic acid (100), freely soluble in methanol, sparingly soluble in ethanol (95), slightly soluble in water, and practically insoluble in acetic anhydride or in ether.

Identification (1) Determine the absorption spectra of solution of Penbutolol Sulfate and Penbutolol Sulfate RS in methanol (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Penbutolol Sulfate and Penbutolol Sulfate RS, previously dried, as directed in the potassium bromide disk method under lnfrared Spectrophotornetry : both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Dissolve 0.1 g of Penbutolol Sulfate in 25 mL of water by warming, and cool: this solution responds to the Qualitative Tests for sulfate.

Melting Point 213 ~ 217 °C.

Specific Optical Rotation $[\alpha]_D^{20}$: -23 ~ -25º (after drying, 0.2 g, methanol, 20 mL, 100 mm).

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Penbutolol Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) ***Arsenic***—Prepare the test solution with 1.0 g of Penbutolol Sulfate according to Method 4, and perform the test (not more than 2 ppm).

(3) ***Related substances***—Dissolve 0.8 g of Penbutolol Sulfate in 10 mL of methanol, and use this solution as the test solution. Pipet 1 mL of the test solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, ethanol (95) and ammonia solution (28) (85 : 12 : 3) to a distance of about 10 cm, and air-dry the plate. Examine under uhraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (0.5 g, 105 °C, 3 hours).

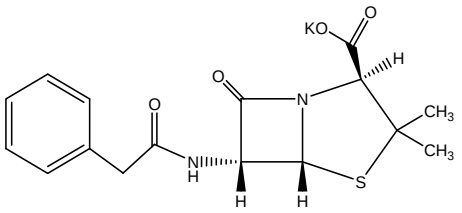
Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 0.8 g of Penbutolol Sulfate, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 68.09 mg of $(C_{18}H_{29}NO_2)_2 \cdot H_2SO_4$

Containers and Storage ***Containers***—Well-closed containers.

Penicillin G Potassium



Benzylpenicillin Potassium $C_{16}H_{17}N_2KO_4S$: 372.48

Potassium(2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[(2-phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [*113-98-4*]

Penicillin G Potassium is the potassium salt of a penicillin substance having antibacterial activity produced by the growth of *Penicillium* species.
Penicillin G Potassium contains not less than 1430 units (potency) and not more than 1630 units (potency) per mg of penicillin G ($C_{16}H_{18}N_2O_4S$: 334.39), calculated on the dried basis. One unit is equivalent to 0.57 μg of penicillin G potassium.

Description Penicillin G Potassium appears as white crystals or crystalline powder.

Penicillin G Potassium is very soluble in water, and slightly soluble in ethanol (99.5).

Identification (1) Determine the absorption spectra of solutions of Penicillin G Potassium and Penicillin G Potassium RS (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Penicllin G Potassium and Penicillin G Potassium RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Penicillin G Potassium responds to the Qualitative Tests (1) for potassium salt.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{20}$: +270 ~ +300° (1 g calculated on the dried basis, water, 50 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g of Penicillin G Potassium in 100 mL of water is between 5.0 and 7.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Penicillin G Potassium in 10 mL of water: the solution is clear and colorless to light yellow.

(2) ***Heavy metals***—Proceed with 2.0 g of Penicillin G Potassium according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Penicillin G Potassium according to Method 4, and perform the test. To 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) in a porcelain crucible add 1 mL of hydrogen peroxide (30), and fire the ethanol to burn (not more than 2 ppm).

(4) ***Related substances***—Weigh accurately 40 mg of Penicillin G Potassium, add water to make exactly 20 mL, and use this solution as the test solution. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method: the area of each peak other than penicillin G from the test solution is not larger than the peak aera of penicillin G from the standard solution, and the total area of the peaks other than penicillin G from the test solution is not larger than 3 times the peak area of penicillin G from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Adjust the pH of a mixture of diammonium hydrogen phosphate solution (33 in 5000) and acetonitrile (19 : 6) to 8.0 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of penicillin G is about 7.5 minutes.

Time span of measurement: About 5 times as long as the retention time of penicillin G

System suitability

Test for required detectability: Pipet 10 mL of the standard solution, and add water to make exactly 100 mL. Confirm that the peak area of penicillin obtained from 20 μL of this solution is equivalent to 7 to 13 % of that from the standard solution.

System performance: Dissolve 40 mg of Penicillin G Potassium in 20 mL of water. Separately, dissolve 10 mg of methyl paraoxybenzoate in 20 mL of acetonitrile. To 1 mL of this solution add water to make 20 mL. To 1 mL each of these solutions add water to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, penicillin G and methyl paraoxybenzoate are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 5 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of penicillin G is not more than 2.0 %.

Loss on Drying Not more than 1.0 % (3 g, reduced pressure not exceeding 0.67 kPa, 60 °C, 3 hours).

Sterility Test It meets the requirement, when Penicillin G Potassium is used in a sterile preparation.

Bacterial Endotoxins Less than 0.01 EU/100 units (potency) of penicillin G, when Penicillin G Potassium is used in a sterile preparation.

Heat Stability Test Weigh accurately about 50000 units (potency) [A unit] of Penicillin G Potassium, according to the result of assay, allow to stand at 100 ± 1 °C for 4 days, and determine a potency [B unit] by reassay (loss in potency: not more than 10 %).

$$\text{Ratio [\%] of loss in potency} = \{([\text{A unit}] - [\text{B unit}]) \times 100\} / [\text{A unit}]$$

Content of Penicillin G Potassium (as by mass ratio) Weigh accurately 60 to 70 mg of Penicillin G Potassium, transfer into stoppered centrifuge tube, add 2.0 mL of water to dissolve, allow to cool to 0 ~ 5 °C, add 2.0 mL of amylacetate TS for penicillin, previously cool to 0 ~ 5 °C, and 0.5 mL of diluted phosphoric acid (1 in 5), shake vigorously to mix for 15 seconds, and centrigure for 20 seconds to separate into two layers.

Take the amylacetate layer (about 1.7 ~ 1.8 mL) by using a 2 mL syringe with needle, and filter through glass filter (diameter about 10 mm) with 0.1 g of sodium sulfate anhydrous. The filter unit is kept cool with ice. Pipet 1.0 mL of this solution, add 0.5 mL of *N*-ethylpiperidine TS for penicillin and 1.0 mL of acetone TS for penicillin, and transfer into a 15 x 15 mm flat-botom test tube, previously weighed the mass. Perform the prodcedure within 3 minutes from acidifying the test solution to transferring the filtrate. Place the above test tube in stoppered weighing bottle, close stopper, and allow to stand at 0 to 8 °C for 2 hours. Filter under suction the precipitate through glass filter (G4, diameter about 10 mm), previously weighed the mass, and wash with 1 mL of acetone TS (0 to 8 °C) for penicillin by using a syringe. Place the glass filter in the test tube, dry in vacuum for 1 hour, and weigh the mass (not less than 85.0 %). Calculate the ratio by using the following equation.

$$\text{Amount [\%] of penicillin G potassium} = \frac{\left[\text{Amount [mg] of the precipitate of N - ethylpiperidine penicillin}\right] \times 166.5}{\text{Amount [mg] of Penicillin G Potassium taken}}$$

Assay Weigh accurately about 60 mg of Penicillin G Potassium, dissolve in 1 % phosphate buffer solution, pH 6.0 to make a solution so that each mL contains 2000 units (potency), and use this solution as the test solution. Separately, weigh accurately about 40 mg of Penicillin G Potassium RS, dissolve in 1 % phosphate buffer, pH 6.0 to make a solution so that each mL contains 2000 units (potency), and use this solution as the standard solution. Pipet 2 mL each of the test solution and the standard solution, transfer into a 100 mL flask for iodine titration, add 2.0 mL of 1 mol/L sodium hydroxide TS, allow to stand for 15 minutes, add 2.0 mL of diluted hydrochloric acid (1 in 10) and 10.0 mL of 0.01 mol/L iodine VS and allow to stand for 15 minutes, if necessary, add about 5 mL of carbon tetrachloride and shake. Pipet 2 mL each of the test solution and the standard solution, add exactly 10.0 mL of 0.01 mol/L iodine VS, titrate each of these solutions in the same manner as the above except 15 minute-standing to perform a blank determination, and make any necessary correction. Determine the consumed amounts (mL) of 0.01 mol/L iodine VS, V_T and V_S, of the test solution and the standard solution, respectively.

$$\text{Potency of Penicillin G Potassium} = \text{Potency of Penicillin G Potassium RS} \times \frac{V_T}{V_S}$$

Containers and Storage ***Containers***—Tight containers.

Penicillin G Potassium for Injection

Penicillin G Potassium for Injection is a preparation for injection, which is dissolved before use.
Penicillin G Potassium for Injection contains not less than 93.0 % and not more than 107.0 % of the labeled units of penicillin G potassium ($C_{16}H_{17}KN_2O_4S$: 372.48).

Method of Preparation Prepare as directed under Injections, with Penicillin G Potassium.

Description Penicillin G Potassium for Injection appears as white crystals or crystalline powder.

Identification Proceed as directed in the Identification (2) under Penicillin G Potassium.

Crystallinity Test It meets the requirement.

pH Dissolve an amount of Penicillin G Potassium for Injection, equivalent to 100000 units of penicillin G potassium, in 10 mL of water: the pH of this solution is between 5.0 and 7.5.

Purity ***Clarity of solution***—Dissolve an amount of Penicillin G Potassium for Injection, equivalent to 1.0×10^6 units of penicillin G potassium according to the labeled amount, in 10 mL of water: the solution is clear, and the absorbance of this solution, determined at 400 nm as directed under Ultraviolet-visible Spectrophotometry, is not more than 0.10.

Loss on Drying Not more than 1.2 % (3 g, reduced pressure not exceeding 0.67 kPa, 60 °C, 3 hours).

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 1.25×10^{-4} EU/unit of penicillin G.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 containers of Penicillin G Potassium for Injection. Weigh accurately a portion of the contents, equivalent to about 6 × 104 units of penicillin G potassium, dissolve in water to make exactly 20 mL, and use this solution as the test solution. Separately, weigh accurately about 6 × 104 units of Penicillin G Potassium RS, dissolve in water to make exactly 20

mL, and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of penicillin G in each solution.

$$\text{Amount (units) of penicillin G potassium } (C_{16}H_{17}KN_2O_4S) = \text{Amount [μg (potency)] of Penicillin G Potassium RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Adjust the pH of a mixture of diammonium hydrogens phosphate solution (33 in 5000) and acetonitrile (19 : 6) to 8.0 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of penicillin G is about 7.5 minutes.

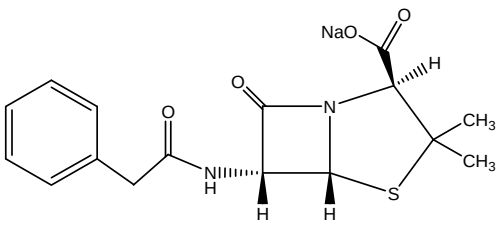
System suitability

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of benzylpenicillin are not less than 6000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of penicillin G potassium is not more than 1.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Penicillin G Sodium



Benzylpenicillin Sodium $C_{16}H_{17}N_2NaO_4S$: 356.37

Sodium(2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[(2-phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [*69-57-8*]

Penicillin G Sodium is the sodium salt of a penicillin substance having antibacterial activity produced by the growth of *Penicillium* species.

Penicillin G Sodium contains not less than 1500 units (potency) and not more than 1750 units (potency) per mg of penicillin G ($C_{16}H_{18}N_2O_4S$: 334.39), calculated on the dried basis.

Description Penicillin G Sodium appears as white crystals or crystalline powder, is odorless or has a little bit of a characteristic odor.

Penicillin G Sodium is very soluble in water, and slightly soluble in acetone.

Penicillin G Sodium is very soluble in Isotonic Sodium Chloride Injection and in Dextrose Injection Solution.

Identification (1) Determine the infrared spectra of Penicillin G Sodium and Penicillin G Sodium RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Penicillin G Sodium responds to the Qualitative Tests (1) for sodium salt.

Crystallinity Test It meets the requirement.

pH The pH of a solution obtained by dissolving 0.6 g of Penicillin G Sodium in 10 mL of water is between 5.0 and 7.5.

Loss on Drying Not more than 1.5 % (0.1 g, in vacuum, 60 °C, 3 hours).

Sterility Test It meets the requirement, when Peniclin G Sodium is used in a sterile preparation.

Bacterial Endotoxins Less than 0.01 EU/100 units (potency) of penicillin G, when Penicillin G Sodium is used in a sterile preparation.

Heat Stability Test Perform the test according to Heat stability test under Penicillin G Potassium (loss in potency: not more than 10 %).

Content of Penicillin G Sodium (as by mass ratio) Perform the test according to Content of penicillin G potassium under Penicillin G Potassium (not less than 85.0 %). Calculate a ratio by using the following equation.

$$\text{Amount [\%] of penicillin G sodium} = \frac{\left[\text{Amount [mg] of the precipitate of N - ethylpiperidine penicillin}\right] \times 159.3}{\text{Amount [mg] of Penicillin G Sodium taken}}$$

Assay Weigh accurately about 10 mg (potency) of Penicillin G Sodium according to the labeled potency, dissolve in the mobile phase to make exactly 100 mL,

and use this solution as the test solution. Separately, weigh accurately about 10 mg of Penicillin G Sodium RS, dissolve in the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of penicillin G sodium in the test solution and standard solution.

$$\text{Amount (units) of penicillin G sodium } (C_{16}H_{17}N_2NaO_4S) = \text{Amount [units (potency)] of Penicillin G Sodium RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm)

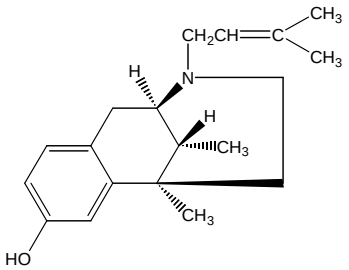
Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of 0.01 mol/L potassium dihydrogen phosphate solution and methanol (3 : 2)

Flow rate: 1.0 mL/minute

Containers and Storage ***Containers***—Tight containers.

Pentazocine



and enantiomer

$C_{19}H_{27}NO$: 285.42

(2*RS*,6*RS*,11*RS*)-6,11-Dimethyl-3-(3-methylbut-2-en-1-yl)-1,2,3,4,5,6-hexahydro-2,6-methanobenzo[d]azocin-8-ol [*359-83-1*]

Pentazocine, when dried, contains not less than 99.0 % and not more than 101.0 % of pentazocine ($C_{19}H_{27}NO$).

Description Pentazocine is a white to pale yellowish white, crystalline powder and is odorless.
Pentazocine is freely soluble in acetic acid (100) or in chloroform, soluble in ethanol (95), sparingly soluble in ether and practically insoluble in water.

Identification (1) Take 1 mg of Pentazocine, add 0.5 mL of formaldehyde-sulfuric acid TS: a deep red color is observed and it changes to grayish brown immediately.

(2) Dissolve 5 mg of Pentazocine in 5 mL of sulfuric acid, add 1 drop of iron (III) chloride TS and heat in a water-bath for 2 minutes: the color of the solution changes from pale yellow to deep yellow. Shake the solution with 1 drop of nitric acid: the solution remains yellow in color.

(3) Determine the absorption spectra of solutions of Pentazocine and Pentazocine RS in 0.01 mol/L hydrochloric acid TS (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting Point 150 ~ 158 ºC.

Absorbance $E_{1cm}^{1\%}$ (278 nm): 67.5 ~ 71.5 (after drying, 0.1 g, 0.01 mol/L hydrochloric acid TS, 1000 mL).

Purity (1) ***Clarity and color of solution***—Dissolve 0.1 g of Pentazocine in 20 mL of 0.1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Pentazocine according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Pentazocine according to Method 3 and perform the test with a solution of magnesium nitrate in ethanol (95) (1 in 10) (not more than 2 ppm).

(4) ***Related substances***—Dissolve 0.20 g of Pentazocine in 10 mL of chloroform and use this solution as the test solution. Pipet 1 mL of the test solution, add chloroform to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and isopropylamine (94 : 3 : 3) to a distance of about 13 cm and air-dry the plate. Allow to stand for 5 minutes in iodine vapor: the spot other than the principal spot from the test solution is not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, P_2O_5, 60 ºC, 5 hours).

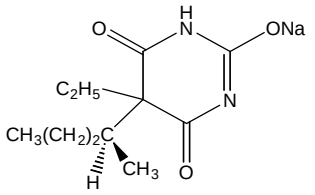
Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 0.5 g of Pentazocine, previously dried, dissolve in 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (indicator: 2 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 28.542 mg of $C_{19}H_{27}NO$

Containers and Storage ***Containers***—Well-closed containers.

Pentobarbital Sodium



$C_{11}H_{17}N_2NaO_3$: 248.25

Sodium 5-ethyl-4,6-dioxo-5-(pentan-2-yl)-1,4,5,6-tetrahydropyrimidin-2-olate [*57-33-0*]

Pentobarbital Sodium contains not less than 98.5 % and not more than 101.0 % of pentobarbital sodium ($C_{11}H_{17}N_2NaO_3$), calculated on the dried basis.

Description Pentobarbital Sodium is a white crystalline granule or powder, is odorless or has a slight characteristic odor and has a slightly bitter taste.
Pentobarbital Sodium is very soluble in water, soluble in ethanol (95) and practically insoluble in ether.
The solution of Pentobarbital Sodium is decomposed on being allowed to stand and the decomposition is accelerated by heating.

Identification (1) Determine the absorption spectra of the test solution and the standard solution obtained in Assay, as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Ignite about 0.2 g of Pentobarbital Sodium and dissolve the residue with acid: the solution responds to the Qualitative Tests for sodium salt.

pH Dissolve 1.0 g of Pentobarbital Sodium in 100 mL of water: the pH of this solution is between 9.8 and 11.0.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Pentobarbital Sodium in 10 mL of freshly boiled and cooled water and allow to stand for 1 minute: the solution is clear and there is no insoluble material.

(2) ***Isomer***—Dissolve about 300 ± 5 mg of Pentobarbital Sodium in 5 mL of water and 300 ± 5 mg of 4-nitrobenzyl bromide in 10.0 mL of ethanol (95). Combine the two solutions, mix by shaking, then reflux for 30 minutes, cool to 25 ºC and filter by suction. Wash the residue with four 5 mL volumes of water, transfer as completely as possible to a small flask, add 25 mL of ethanol (95) and reflux for 10 minutes: the solid dissolves completely. Cool the solution and filter by suction: the collected solid, after being dried at 105 ºC for 30 minutes, melts between 136 and 146 ºC .

(3) ***Heavy metals***—Dissolve 2.0 g of Pentobarbital Sodium in 50 mL of water. Add slowly, with vigorous shaking, 5 mL of dilute hydrochloric acid and warm for 2 minutes in a water-bath with occasional shaking. Cool, add 25 mL of water with shaking and filter. Discard the first 10 mL of the filtrate, take 40 mL of the subsequent filtrate, add 1 drop of phenolphthalein TS, add ammonia TS drop-wise until a pale red color is observed, add 2 mL of dilute acetic acid, add water to make 50 mL and use this solution as the test solution. Perform the test. Prepare the control solution as follows: add 2.5 mL of dilute acetic acid and 1 drop of phenolphthalein TS, add ammonia TS drop-wise until a pale red color is observed, add 2 mL of dilute acetic acid, 3.0 mL of standard lead solution and water to make 50 mL (not more than 30 ppm).

(4) ***Related substances***—Weigh accurately about 0.11 g of Pentobarbital Sodium, add 80 mL of the mobile phase, sonicate to dissolve, add the mobile p hase to make exactly 100 mL, and use this solution as the test solution. Separately, weigh accurately about 0.1 mg of Pentobarbital RS, dissolve in the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions. Determine the peak areas of each solution by the automatic integration method, and calculate the amount of each related substance in the test solution by the following equation: pentobarbital related substance I {6-imino-5-ethyl-5-(1-methylbutyl)barbituric acid} is not more than 0.2 %, pentobarbital related substance II {5-ethyl-5-(1-ethylpropyl)barbituric acid} is not more than 0.1 %, and pentobarbital related substance III {5-ethyl-5-(1,3-dimethylbutyl)barbituric acid} is not more than 0.3 %. The amount of any other related substance is not more than 0.1 %, and the total amount of related substances is not more than 0.5 %. Use the peak areas of related substances I and III after dividing by their relative response factors, 1.5 and 0.9, respectively.

$$\text{Amount (\%) of related substances} = \frac{248.25}{226.27} \times 10000 \times \frac{C}{W} \times \frac{A_i}{A_S}$$

248.25: Molecular weight of pentobarbital sodium
226.27: Molecular weight of pentobarbital
C: Concentration (mg/mL) of pentobarbital in the standard solution
W: Amount (mg) of the sample, calculated on the anhydrous basis
A_i: Peak area of each related substance obtained from the test solution
A_S: Peak area of pentobarbital obtained from the standard solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 214 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Flow rate: About 1.0 mL/minute

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the relative retention times of pentobarbital related substances I, II and III with respect to pentobarbital are about 0.39, about 0.93, and about 1.5, respectively. The capacity factor (*k'*) is not less than 2.5, and the number of theoretical plates and symmetry factor of the peak of pentobarbital are not less than 15000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pentobarbital is not more than 15.0 %.

Loss on Drying Not more than 3.5 % (1 g, 105 ºC, 6 hours).

Assay Weigh accurately about 25 mg of Pentobarbital Sodium, previously dried and add diluted ammonia solution (28) (1 in 200) to make exactly 50 mL. Pipet 2 mL of this solution, add diluted ammonia solution (28) (1 in 200) to make exactly 100 mL and use this solution as the test solution. Separately, weigh accurately about 0.1 g of Pentobarbital RS and dissolve in diluted ammonia solution (28) (1 in 200), to make exactly 100 mL. Pipet 1 mL of this solution, add diluted ammonia solution (28) (1 in 200) to make exactly 100 mL, use this solution as a standard solution. Determine the absorbances of the test solution and the standard solution, A_T and A_S, at 240 nm as directed under Ultraviolet-visible Spectrophotometry, using diluted ammonia solution (28) (1 in 200) as a blank.

$$\text{Amount (mg) of pentobarbital sodium } (C_{11}H_{17}N_2NaO_3) = \text{Amount (mg) of Pentobarbital RS} \times \frac{A_T}{A_S} \times 1.097 \times \frac{1}{4}$$

Containers and Storage ***Containers***—Tight containers.

Pentobarbital Sodium Capsules

Pentobarbital Sodium Capsules contain not less than 92.5 % and not more than 107.5 % of the labeled amount of pentobarbital sodium ($C_{11}H_{17}N_2NaO_3$: 248.25).

Method of Preparation Prepare as directed under Capsules, with Pentobarbital Sodium.

Identification Dissolve a portion of Pentobarbital Sodium Capsules, equivalent to about 0.1 g of pentobarbital sodium according to the labeled amount, in 15 mL of water in a separatory funnel, add 2 mL of hydrochloric acid, shake well and extract the liberated pentobarbital with five 25 mL volumes of chloroform. Filter each extract through a suitable filter. Evaporate the combined chloroform extract to dryness in a water-bath with the aid of a current of air, add 10 mL of ether and evaporate to dryness again. Recrystallize from hot ethanol and dry the residue at 105 ºC for 30 minutes. With the residue so obtained, proceed as directed in the Identification (1) under Pentobarbital Sodium.

Dissolution Test Perform the test with 1 capsule of Pentobarbital Sodium Capsules at 100 revolutions per minute according to Method 1 under the Dissolution Test, using 900 mL of water as the dissolution solution. Take the dissolved solution after 45 minutes from starting of the test and filter. Add freshly prepared diluted ammonia solution (28) (1 in 20) to obtain a solution having known concentration of about 10 μg of pentobarbital per mL and use this solution as the test solution. Separately, weigh accurately a portion of Pentobarbital RS, dissolve in freshly prepared diluted ammonia solution (28) (1 in 20) to obtain a solution having known concentration of about 10 μg of pentobarbital per mL and use this solution as the standard solution. The concentration of the standard solution, multiplied by 1.097, represents the equivalent amount of Pentobarbital Sodium. Determine the absorbances of the test solution and the standard solution at 240 nm as directed under Ultraviolet-visible Spectrophotometry using diluted ammonia solution (28) (1 in 20) as the blank.

The dissolution rate of Pentobarbital Sodium Capsules in 45 minutes is not less than 75 %.

Uniformity of Dosage Units It meets the requirement when the content uniformity test is performed according to the following procedure.

Transfer the contents of 1 capsule of Pentobarbital Sodium Capsules with 5 mL of ethanol to a 250-mL volumetric flask, add 10 mL of freshly prepared diluted ammonia solution (28) (1 in 200) and dilute with immediately the same solution to make 250 mL. Mix by shaking and filter, if necessary. Discard the first 20 mL of the filtrate and dilute the subsequent filtrate with diluted ammonia solution (28) (1 in 200) to obtain a solution having known concentration of 10 μg of pentobarbital sodium per mL and use this solution as the test solution. Separately, weigh accurately a suitable amount of Pentobarbital RS, dissolve in diluted ammonia solution (28) (1 in 200) to obtain a solution having known concentration of 10 μg of pentobarbital sodium per mL and use this solution as the standard solution. Determine the absorbances of the test solution and the standard solution at 240 nm as directed under Ultraviolet-visible Spectrophotometry using diluted ammonia solution (28) (1 in 20) as the blank.

Amount (mg) of pentobarbital sodium ($C_{11}H_{17}N_2NaO_3$) in 1 capsule = Labeled amount (mg) of Pentobarbital Sodium in 1 capsule $\times \frac{C_S}{C_T} \times \frac{A_T}{A_S} \times 1.097$

C_S: Concentration (μg/mL) of pentobarbital in the standard solution

C_T: Concentration (μg/mL) of pentobarbital sodium in the test solution, calculated on the basis of the labeled am ount per capsule and the extent of dilution

Assay Weigh accurately the contents of not less than 20 Pentobarbital Sodium Capsules, mix uniformly the contents and transfer accurately a portion of the powder, equivalent to about 50 mg of pentobarbital sodium ($C_{11}H_{17}N_2NaO_3$), to a separatory funnel. Add 15 mL of water and 1 mL of hydrochloric acid, shake well and extract with five 25 mL volumes of chloroform. Filter the extracts through about 15 g of anhydrous sodium sulfate that is supported on a funnel by a small pledget of glass wool. Collect the combined filtrate in a volumetric flask, wash the sodium sulfate with 15 mL of chloroform, collecting the washing with the filtrate and add chloroform to make exactly 100 mL. Mix 2.0 mL of this solution with 1.0 mL of the internal standard solution and use this solution as the test solution. Separately, weigh accurately about 45 mg of pentobarbital RS, previously dried at 105 ºC for 2 hours, dissolve in chloroform to make exactly 100 mL, mix 2.0 mL of this solution with 1.0 mL of the internal standard solution and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Gas Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of pentobarbital to that of the internal standard for the test solution and the standard solution, respectively.

Amount (mg) of pentobarbital sodium ($C_{11}H_{17}N_2NaO_3$) = Amount (mg) of Pentobarbital RS $\times \frac{Q_T}{Q_S} \times 1.097$

Internal standard solution—A solution of n-tricosane in chloroform (4 in 10000).

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A glass column, about 4 mm in internal diameter and about 0.9 m in length, packed with siliceous earth for gas chromatography (149 μm to 177 μm in particle diameter), coated with polyamide at the ratio of 3 %.

Column temperature: 200 ± 10 ºC.

Injection point and detector temperature: About 225 ºC .

Carrier gas: Nitrogen.

Flow rate: 60 to 80 mL/minute.

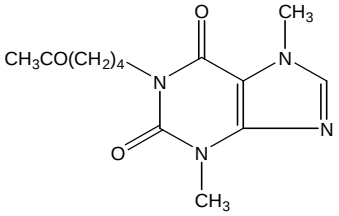
System suitability

System performance: When the procedure is run with 5 μL of the standard solution according to the above operating conditions, n-tricosane and pentobarbital are eluted in this order with the resolution between their peaks being not less than 2.3 and with a symmetry factor being not more than 2.0.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution according to the above operating conditions: the relative standard deviation of the ratios of the peak area is not more than 1.5 %.

Containers and Storage ***Containers***—Tight containers.

Pentoxifylline



$C_{13}H_{18}N_4O_3$: 278.31

3,7-Dimethyl-1-(5-oxohexyl)-1*H*-purine-2,6 (3*H*,7*H*)-dione [*6493-05-6*]

Pentoxifylline, when dried, contains not less than 98.0 % and not more than 102.0 % of pentoxifylline ($C_{13}H_{18}N_4O_3$).

Description Pentoxifylline appears as white, crystalline powder, has a characteristic odor and a bitter taste. Pentoxifylline is freely soluble in acetic acid (100), soluble in water, in methanol, in ethanol or in acetic anhydride and slightly soluble in ether.

Identification (1) Take 10 mg of Pentoxifylline, add 10 drops of hydrogen peroxide TS and 1 drop of hydrochloric acid and evaporate on a water-bath to dryness: a yellow-red color is observed in the residue. Hold the residue over a vessel containing 2 to 3 drops of ammonia TS: the color changes to red-purple and on the addition of 2 to 3 drops of sodium hydroxide TS, the color disappears.

(2) Dissolve 0.1 g of Pentoxifylline in 10 mL of 6 mol/L acetic acid TS by warming and add, while warming, a solution of 70 mg of 2,4-dinitrophenylhydrazine in 10 mL of diluted acetic acid (1 in 2). After cooling, filter and collect the yellow precipitate produced, wash with water, then with a small quantity of cold ethanol (95) and dry at 80 ºC for 1 hour: it melts between 199 and 201 ºC.

(3) Determine the absorption spectra of solutions of Pentoxifylline and Pentoxifylline RS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry:

both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared spectra of Pentoxifylline and Pentoxifylline RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 104 ~ 106 ºC.

pH The pH of a solution of Pentoxifylline (1 in 100) is between 5.0 and 7.5.

Absorbance $E_{1cm}^{1\%}$ (274 nm): 360 ~ 376 (10 mg, water, 1000 mL).

Purity (1) ***Acid***—Dissolve 1 g of Pentoxifylline in 50 mL of freshly boiled and cooled water, and add 1 drop of bromothymol blue TS. Titrate with 0.01 mol/L sodium hydroxide TS until the color of the solution changes: not more than 0.2 mL is consumed.

(2) ***Chloride***—Dissolve 2.0 g of Pentoxifylline in 80 mL of hot water, cool rapidly to 20 ºC, add water to make 100 mL. To 40 mL of this solution, add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS, add 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.011 %).

(3) ***Sulfate***—To 40 mL of the solution obtained in (1), add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS, add 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.024 %).

(4) ***Heavy metals***—Proceed with 2.0 g of Pentoxifylline according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(5) ***Arsenic***—Prepare the test solution with 2.0 g of Pentoxifylline according to Method 3 and perform the test (not more than 1 ppm).

(6) ***Related substances***—Dissolve 0.10 g of Pentoxifylline in 5.0 mL of methanol and use this solution as the test solution. Pipet 1 mL of the test solution and add methanol to make exactly 100 mL. Pipet 3 mL of this solution, add methanol to make exactly 10 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 20 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and methanol (17 : 3) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, P_2O_5, 60 ºC, 3 hours).

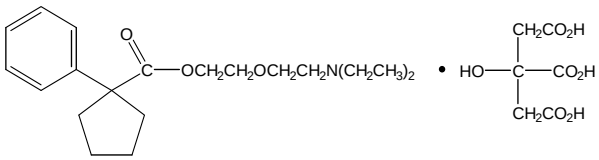
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.3 g of Pentoxifylline, previously dried, dissolve in 40 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 27.831 mg of $C_{13}H_{18}N_4O_3$

Containers and Storage ***Containers***—Tight containers.

Pentoxyverine Citrate



$C_{20}H_{31}NO_3 \cdot C_6H_8O_7$: 525.60

2-[2-(Diethylamino)ethoxy]ethyl1-phenylcyclopentane-1-carboxylate 2-hydroxypropane-1,2,3-tricarboxylate [*23142-01-0*]

Pentoxyverine Citrate, when dried, contains not less than 98.5 % and not more than 101.0 % of pentoxyverine citrate ($C_{20}H_{31}NO_3 \cdot C_6H_8O_7$).

Description Pentoxyverine Citrate is a white, crystalline powder.
Pentoxyverine Citrate is very soluble in acetic acid (100), freely soluble in water and in ethanol (95) and practically insoluble in ether.

Identification (1) Dissolve 0.1 g of Pentoxyverine Citrate in 10 mL of water and add 10 mL of Reinecke salt TS: a pale red precipitate is produced.

(2) Determine the infrared spectra of Pentoxyverine Citrate and pentoxyverine citrate RS, previously dried, as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Pentoxyverine Citrate (1 in 10) responds to the Qualitative Tests (1) and (2) for citrate.

Melting Point 92 ~ 95 °C.

Purity (1) ***Clarity and color of solution***—Dissolve

1.0 g of Pentoxyverine Citrate in 10 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 2.0 g of Pentoxyverine Citrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Pentoxyverine Citrate according to Method 3 and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 0.20 g of Pentoxyverine Citrate in 10 mL of ethanol (95) and use this solution as the test solution. Pipet 1 mL of the test solution, add ethanol (95) to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 15 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Immediately after air-drying, develop the plate with a mixture of chloroform, methanol, ethyl acetate and ammonia solution (28) (25 : 10 : 10 : 1) to a distance of about 10 cm and air-dry the plate. Allow to stand in iodine vapor for 10 minutes: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, P_2O_5, 60 °C, 4 hours).

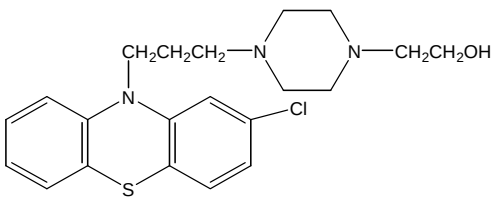
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.5 g of Pentoxyverine Citrate, previously dried, dissolve in 30 mL of acetic acid (100), add 30 mL of acetic anhydride and titrate with 0.1 mol/L of perchloric acid VS until the color of the solution changes from purple through blue-green to green (indicator: 3 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 52.56 mg of $C_{20}H_{31}NO_3 \cdot C_6H_8O_7$

Containers and Storage ***Containers***—Well-closed containers.

Perphenazine



$C_{21}H_{26}ClN_3OS$: 403.97

2-{4-[3-(2-Chloro-10*H*-phenothiazin-10-yl)propyl]piperazin-1-yl}ethan-1-ol [*58-39-9*]

Perphenazine, when dried, contains not less than 98.5 % and not more than 101.0 % of perphenazine ($C_{21}H_{26}ClN_3OS$).

Description Perphenazine appears as white to pale yellow crystals or crystalline powder, is odorless and has a bitter taste.
Perphenazine is freely soluble in methanol or in ethanol (95), soluble in glacial acetic acid, sparingly soluble in ether and practically insoluble in water.
Perphenazine dissolves in dilute hydrochloric acid.
Perphenazine is gradually colored by light.

Identification (1) Dissolve 5 mg of Perphenazine in 5 mL of sulfuric acid: a red color, changing to deep red-purple upon warming, is observed.

(2) Dissolve 0.2 g of Perphenazine in 2 mL of methanol, add this solution to 10 mL of a warm solution of 2,4,6-trinitrophenol in methanol (1 in 25) and allow to stand for 4 hours. Filter and collect the crystals, wash with a small volume of methanol and dry at 105 ºC for 1 hour: the crystals so obtained melt between 237 ºC and 244 ºC (with decomposition).

(3) Determine the absorption spectra of solutions of Perphenazine and Perphenazine RS in 0.1 mol/L hydrochloric acid VS (1 in 200000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths. Add 10 mL of water to another 10 mL each of these solutions and determine the absorption spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Perform the test with Perphenazine as directed under the Flame Coloration Test (2): a green color is observed.

Melting Point 95 ~ 100 ºC.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Perphenazine according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Perform the test in the current of nitrogen in light-resistant containers under the protection from sunlight. Dissolve 0.10 g of Perphenazine in 10 mL of ethanol (95) and use this solution as the test solution. Pipet 1 mL of the test solution and add ethanol (95) to make exactly 10 mL. Pipet 1 mL of this solution, add ethanol (95) to make exactly 20 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol and 1 mol/L ammonia TS (5 : 1) to a distance of about 12 cm and air-

dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot from the test solution is not more intense than that from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, P_2O_5, 65 ºC, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.4 g of Perphenazine, previously dried, dissolve in 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue-purple to blue-green (indicator: 3 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 20.198 mg of $C_{21}H_{26}ClN_3OS$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Perphenazine Tablets

Perphenazine Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of perphenazine ($C_{21}H_{26}ClN_3OS$: 403.98).

Method of Preparation Prepare as directed under Tablets, with Perphenazine.

Identification (1) Shake well a portion of powdered Perphenazine Tablets, equivalent to 25 mg of Perphenazine according to the labeled amount, with 10 mL of methanol and filter. Evaporate 2 mL of the filtrate in a water-bath to dryness. With the residue, proceed as directed in the Identification (1) under Perphenazine.

(2) Add 5 mL of the filtrate obtained in the Identification (1) to 10 mL of a warm solution of 2,4,6-trinitro-phenol in methanol (1 in 25) and proceed as directed in the Identification (2) under Perphenazine.

(3) Determine the absorption spectrum of the filtrate obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry: it exhibits an absorption maximum between 309 nm and 313 nm. Add 30 mL of methanol to another 10 mL of the filtrate and determine the absorption spectrum: it exhibits an absorption maximum between 256 nm and 260 nm.

Dissolution Test Perform the test with 1 tablet of Perphenazine Tablets at 100 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of diluted phosphate buffer solution, pH 6.8 (1 in 2) as the dissolution solution. Take 30 mL or more of the dissolved solution after 90 minutes from start of the test and filter through a membrane filter with a pore size of not more than 0.8 μm. Discard the first 10 mL of the filtrate and use the subsequent filtrate as the test solution. Separately, weigh accurately about 10 mg of Perphenazine RS, previously dried in vacuum with P_2O_5 at 65 ºC for 4 hours, dissolve in 5 mL of 0.1 mol/L hydrochloric acid TS and add diluted phosphate buffer solution, pH 6.8, (1 in 2) to make exactly 250 mL. Pipet 5 mL of this solution, add diluted phosphate buffer solution, pH 6.8, (1 in 2) to make exactly 50 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 255 nm as directed under Ultraviolet-visible Spectrophotometry.

The dissolution rate of Perphenazine Tablets in 90 minutes is not less than 70 %.

$$\text{Dissolution rate (\%) with respect to the labeled amount of Perphenazine } (C_{21}H_{26}ClN_3OS) = W_S \times \frac{A_T}{A_S} \times \frac{1}{C} \times 36$$

W_S: Amount (mg) of Perphenazine RS

C: Labeled amount (mg) of Perphenazine ($C_{21}H_{26}ClN_3OS$) in 1 tablet.

Uniformity of Dosage Units It meets the requirement when the content uniformity test is performed according to the following procedure.

Disintegrate 1 Tablet of Perphenazine Tablets by shaking with 5 mL of water, shake well with 70 mL of methanol and add methanol to make exactly 100 mL. Centrifuge this solution, pipet x mL of the clear supernatant liquid, add methanol to make exactly V mL of a solution containing about 4 g of perphenazine ($C_{21}H_{26}ClN_3OS$) in each mL and use this solution as the test solution. Separately, weigh accurately about 10 mg of Perphenazine RS, previously dried in vacuum over P_2O_5 at 65 ºC for 4 hours, dissolve in methanol to make exactly 250 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 258 nm as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of Perphenazine } (C_{21}H_{26}ClN_3OS) = \text{Amount (mg) of Perphenazine RS} \times \frac{A_T}{A_S} \times \frac{V}{25} \times \frac{1}{x}$$

Assay Weigh accurately and powder not less than 20 Perphenazine Tablets. Weigh accurately a portion of the powder, equivalent to about 5 mg of perphenazine ($C_{21}H_{26}ClN_3OS$), dissolve in methanol to make exactly 100 mL and filter. Discard the first 10 mL of the filtrate, pipet 10 mL of the subsequent filtrate and 5 mL of the internal standard solution, add methanol to make exactly 100 mL and use this solution as a test solution. Separately, weigh accurately about 25 mg of Perphenazine

RS previously dried in desiccator (in vaccume, P_2O_5, 65 ºC) for 4 hours and dissolve in methanol to make exactly 100 mL. Pipet 2 mL of this solution and 5 mL of internal standard solution, add methanol to make exactly 100 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of perphenazine to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of perphenazine } (C_{21}H_{26}ClN_3OS) = \text{Amount (mg) of Perphenazine RS} \times \frac{Q_T}{Q_S} \times \frac{1}{5}$$

Internal standard solution—Dissolve 50 mg of anhydrous caffeine RS in methanol to make 50 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (main wavelength: 254 nm)

Column: A stainless steel column, about 4 mm in internal diameter and 15 cm to 30 cm in length, packed with nitrilized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Mobile phase: Dissolve 0.65 g of ammonium acetic acid in 84 mL of water, add 916 mL of methanol.

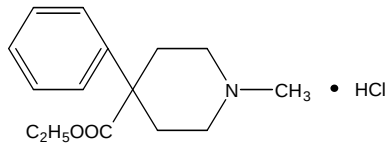
Flow rate: Adjust the flow rate so that the retention time of perpernazine is about 5.5 minutes.

Selection of column: Proceed with 20 μL of this solution according to the above operating conditions, and calculate the resolution. Use a column giving elution of internal standard and Perpernazine in this order with the resolution between their peaks being not less than 3.0.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Pethidine Hydrochloride



Operidine $C_{15}H_{21}NO_2 \cdot HCl$: 283.79

Ethyl 1-methyl-4-phenylpiperidine-4-carboxylate hydrochloride [*50-13-5*]

Pethidine Hydrochloride, when dried, contains not less than 98.0 % and not more than 101.0 % of pethidine hydrochloride ($C_{15}H_{21}NO_2 \cdot HCl$).

Description Pethidine Hydrochloride is a white, crystalline powder.

Pethidine Hydrochloride is very soluble in water or in acetic acid (100), freely soluble in ethanol (95), sparingly soluble in acetic anhydride and practically insoluble in ether.

pH—The pH of a solution dissolved 1.0 g of Pethidine Hydrochloride in 20 mL of water is between 3.8 and 5.8.

Identification (1) Determine the absorption spectra of solutions of Pethidine Hydrochloride and Pethidine Hydrochloride RS (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Pethidine Hydrochloride and Pethidine Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Pethidine Hydrochloride (1 in 50) responds to the Qualitative Tests (2) for chloride.

Melting Point 187 ~ 189 °C.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Pethidine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) ***Sulfate***—Perform the test with 0.20 g of Pethidine Hydrochloride. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.240 %).

(3) ***Related substances***—Dissolve 50 mg of Pethidine Hydrochloride in 20 mL of the mobile phase and use this solution as the test solution. Pipet 1 mL of the test solution, add the mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine each peak area obtained from the test solution and the standard solution by the automatic integration method: the total area of the peaks other than that of pethidine from the test solution is not larger than the peak area of pethidine from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (main wavelength: 257 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (about 5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: Dissolve 2.0 g of sodium lauryl sulfate in 1000 mL of diluted phosphoric acid (1 in 1000),

adjust the pH to 3.0 with sodium hydroxide TS and to 550 mL of this solution, add 450 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of pethidine is about 7 minutes.

System suitability

Test for required detectability: Pipet 2 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of pethidine obtained from 20 μL of this solution is equivalent to 5 to 15 % of that from the standard solution.

System performance: Take 2 mL each of the test solution and a solution of isoamyl parahydroxybenzoate in the mobile phase (1 in 50000) and add the mobile phase to make 10 mL. When the procedure is run with 20 μL of this solution according to the above operating conditions, pethidine and isoamyl parahydroxybenzoate are eluted in this order with the resolution between their peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution according to the above operating conditions: the relative standard deviation of the peak area of pethidine is not more than 2.0 %.

Time span of measurement: About 2 times as long as the retention time of pethidine after the solvent peak.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (0.5 g).

Assay Weigh accurately about 0.5 g of Pethidine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 28.379 mg of $C_{15}H_{21}NO_2 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Pethidine Hydrochloride Injection

Operidine Injection

Pethidine Hydrochloride Injection is an aqueous solution for injection. Pethidine Hydrochloride Injection contains not less than 95.0 % and not more than 105.0 % of the labeled amount of pethidine hydrochloride ($C_{15}H_{21}NO_2 \cdot HCl$: 283.79).

Method of Preparation Prepare as directed under Injections, with Pethidine Hydrochloride.

Description Pethidine Hydrochloride Injection appears as clear, colorless liquid.

Pethidine Hydrochloride Injection is affected by light.

pH—4.0 ~ 6.0.

Identification Take a volume of Pethidine Hydrochloride Injection, equivalent to 0.1 g of pethidine hydrochloride according to the labeled amount and add water to make 200 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits absorption maxima between 250 nm and 254 nm, between 255 nm and 259 nm and between 261 nm and 265 nm.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 6.0 EU/mg of pethidine hydrochloride.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay Measure exactly a volume of Pethidine Hydrochloride Injection, equivalent to about 0.1 g of pethidine hydrochloride ($C_{15}H_{21}NO_2 \cdot HCl$) according to the labeled amount, add exactly 10 mL of the internal standard solution and add the mobile phase to make 50 mL. To 5 mL of this solution, add the mobile phase to make exactly 20 mL and use this solution as the test solution. Separately, weigh accurately about 0.1 g of Pethidine Hydrochloride RS, previously dried at 105 °C for 3 hours, add exactly 10 mL of the internal standard solution and add the mobile phase to make exactly 50 mL. To 5 mL of this solution, add the mobile phase to make 20 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and calculate the ratios, Q_T and Q_S, of the peak area of pethidine to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of pethidine hydrochloride } (C_{15}H_{21}NO_2 \cdot HCl) = \text{Amount (mg) of Pethidine Hydrochloride RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of isoamyl parahydroxybenzoate in the mobile phase (1 in 12500).

Operating conditions

Detector: An ultraviolet absorption photometer (main wavelength: 257 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (about 5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: Dissolve 2.0 g of sodium lauryl sulfate in 1000 mL of diluted phosphoric acid (1 in 1000), adjust the pH to 3.0 with sodium hydroxide TS and to 550 mL of this solution, add 450 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of pethidine is about 7 minutes.

System suitability

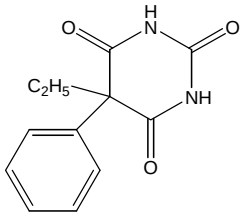
System performance: When the procedure is run with 20 μL of the standard solution according to the above operating conditions, pethidine and the internal standard are eluted in this order with the resolution between their peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution according to the above operating conditions, the relative standard deviation of the ratios of the peak area of pethidine to that of the internal standard material is not more than 1 %.

Containers and Storage ***Containers***—Hermetic containers. Colored containers may be used.

Storage—Light-resistant.

Phenobarbital



$C_{12}H_{12}N_2O_3$: 232.24

5-Ethyl-5-phenyl-1,3-diazinane-2,4,6-trione [*50-06-6*]

Phenobarbital, when dried, contains not less than 99.0 % and not more than 101.0 % of phenobarbital ($C_{12}H_{12}N_2O_3$).

Description Phenobarbital appears as white crystals or crystalline powder, is odorless and has a bitter taste.
Phenobarbital is very soluble in *N,N*-dimethylformamide, freely soluble in ethanol (95), in acetone or in pyridine, soluble in ether and very slightly soluble in water.
Phenobarbital dissolves in sodium hydroxide TS or in ammonia TS.

pH—The pH of a saturated solution of Phenobarbital is between 5.0 and 6.0.

Identification (1) Determine the absorption spectra of solutions of Phenobarbital and Phenobarbital RS in boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6 (1 in 100000), as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Phenobarbital and Phenobarbital RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting Point 175 ~ 179 ºC.

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Phenobarbital in 5 mL of sodium hydroxide TS: the solution is clear and colorless.

(2) ***Chloride***—Dissolve 0.30 g of Phenobarbital in 20 mL of acetone and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test. Prepare the control solution as follows: take 0.30 mL of 0.01 mol/L hydrochloric acid VS, 20 mL of acetone and 6 mL of dilute nitric acid and add water to make 50 mL (not more than 0.035 %)

(3) ***Heavy metals***—Proceed with 1.0 g of Phenobarbital according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Phenylbarbituric acid***—Boil 1.0 g of Phenobarbital with 5 mL of ethanol (95) for 3 minutes: the solution is clear.

(5) ***Related substances***—Dissolve about 0.10 g of Phenobarbital in 100 mL of acetonitrile, and use this solution as the test solution. Pipet 2 mL of the test solution, and add acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method: the area of each peak other than phenobarbital obtained from the test solution is not larger than the peak area of phenobarbital from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and 150 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45 °C

Mobile phase: A mixture of water and acetonitrile (11 : 9)

Flow rate: Adjust the flow rate so that the retention time of phenobarbital is about 5 minutes.

Time span of measurement: About 12 times as long as the retention time of phenobarbital, beginning after the solvent peak.

System suitability

Test for required detectability: Pipet 5 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of phenobarbital obtained from 10 μL of this solution is equivalent to 20 to 30 % of that from the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates is not less than 3000 and the symmetry factor is not more than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of phenobarbital is not more than 3.0 %.

Loss on Drying Not more than 1.0 % (1 g, 105 ºC, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.5 g of Phenobarbital, previously dried, dissolve in 50 mL of *N,N*-dimethylformamide and titrate with 0.1 mol/L potassium hydroxide-ethanol VS until the color of the solution change from yellow to yellow-green (indicator: 1 mL of alizarin yellow GG-thymol-phthalein TS). Perform a blank determination using a mixture of 50 mL of *N,N*-dimethylformamide and 22 mL of ethanol (95) and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 23.224 mg of $C_{12}H_{12}N_2O_3$

Containers and Storage ***Containers***—Well-closed containers.

10 % Phenobarbital Powder

Phenobarbital Powder contains not less than 9.3 % and not more than 10.7 % of phenobarbital ($C_{12}H_{12}N_2O_3$: 232.24).

Method of Preparation

Phenobarbital	100 g
Starch, lactose hydrate, or their mixture	a sufficient quantity
To make	1000 g

Prepare as directed under Powders, with the above ingredients.

Identification (1) Determine the absorption spectrum of the test solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 238 and 242 nm.

(2) To 6 g of 10 % Phenobarbital Powder add 150 mL of ethanol, shake well, and filter. Evaporate the filtrate to about 5 mL on a water bath, add about 50 mL of water to crystallize, filter, and take the crystals. Dry the crystals at 105 °C for 2 hours, and determine the absorption spectra as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Dissolution Test Perform the test with 0.3 g of 10 % Phenobarbital Powder, accurately weighed, at 50 revolutions per minute according to Method 2, using 900 mL of water as the dissolution solution. Take the dissolved solution 30 minutes after the start of the test, filter, take not less than 20 mL, and filter thorugh a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add pH 9.6 boric acid-potassium chloride-sodium hydroxide buffer solution to make exactly 10 mL, and use this solution as the test solution. Separately, weigh accurately about 17 mg of Phenobarbital RS (previously dried at 105 °C for 2 hours), and dissolve in water to make 100 mL. Pipet 5 mL of this solution, and add water to make 25 mL. Pipet 5 mL of this solution, add pH 9.6 boric acid-potassium chloride-sodium hydroxide buffer solution to make exactly 10 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 240 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry, using a mixture of pH 9.6 boric acid-potassium chloride-sodium hydroxide buffer solution and water (2 : 1) as the blank. The dissolution rate of 10 % Phenobarbital Powder, in 30 minutes is not less than 80 %.

Dissolution rate (%) with respect to the labeled amount of phenobarbital ($C_{12}H_{12}N_2O_3$)

$$= \frac{W_S}{W_T} \times \frac{A_T}{A_S} \times \frac{1}{C} \times 180$$

W_S: Amount (mg) of Phenobarbital RS

W_T: Amount (g) of 10 % Phenobarbital Powder taken

C: Labeled amount (mg) of phenobarbital ($C_{12}H_{12}N_2O_3$) in 1 g

Particle Size Distribution Test for Preparations It meets the requirement.

Uniformity of Dosage Units (divided) It meets the requirement.

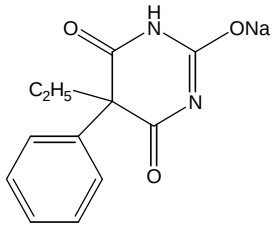
Assay Weigh accurately about 0.2 g of 10 % Phenobarbital Powder, and dissolve in pH 9.6 boric acid-potassium chloride-sodium hydroxide buffer solution

to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 100 mL of pH 9.6 boric acid-potassium chloride-sodium hydroxide buffer solution, and use this solution as the test solution. Separately, weigh accurately about 20 mg of Phenobarbital RS, previously dried at 105 °C for 2 hours, and dissolve in pH 9.6 boric acid-potassium chloride-sodium hydroxide buffer solution to make exactly 100 mL. Pipet 5 mL of this solution, add pH 9.6 boric acid-potassium chloride-sodium hydroxide buffer solution to make 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 240 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry, using pH 9.6 boric acid-potassium chloride-sodium hydroxide buffer solution as the blank.

$$\text{Amount (mg) of phenobarbital } (C_{12}H_{12}N_2O_3) = \text{Amount (mg) of Phenobarbital RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Well-closed containers.

Phenobarbital Sodium



$C_{12}H_{11}N_2NaO_3$: 254.22

Sodium 5-ethyl-4,6-dioxo-5-phenyl-1*H*-pyrimidin-2-olate [*57-30-7*]

Phenobarbital Sodium contains not less than 98.5 % and not more than 101.0 % of phenobarbital sodium ($C_{12}H_{11}N_2NaO_3$), calculated on the dried basis.

Description Phenobarbital Sodium appears as white crystals or white powder, is odorless and has a slightly bitter taste.
Phenobarbital Sodium is very soluble in water, soluble in ethanol (95) and practically insoluble in ether or in chloroform.
Phenobarbital Sodium is hygroscopic and gradually decomposed on being allowed to stand in the humid air.

Identification (1) Dissolve about 50 mg of Phenobarbital Sodium in 15 mL of water in a separatory funnel, add 2 mL of hydrochloric acid, shake and extract with four 25 mL volumes of chloroform. Filter the combined extracts, take 50 mL of filtrate and evaporate on a water-bath with aid of a current air. Add 10 mL of ether, again evaporate and dry the residue at 105 ºC for 2 hours. Determine the infrared spectra of the residue obtained above and Phenobarbital RS, respectively, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Ignite about 0.2 g of Phenobarbital Sodium and dissolve the residue in 10 mL of water. This solution changes red litmus paper to blue and responds to the Qualitative Tests for sodium salt.

(3) The retention time of the principal peak of the chromatogram obtained from the test solution corresponds to that from the standard soution, as obtained in the Assay.

pH The pH of a solution of Phenobarbital Sodium (1 in 10) is between 9.2 and 10.2.

Purity (1) ***Clarity of color of solution***—Dissolve 1.0 g of Phenobarbital Sodium in 10 mL of freshly boiled and cooled water: the solution is colorless and clear after 1 minute.

(2) ***Heavy metals***—Dissolve 2.0 g of Phenobarbital Sodium in 52 mL of water. Add 8 mL of 1 mol/L hydrochloric acid with vigorous shaking and filter. Discarding the first 5 mL of the filtrate and dilute 20 mL of the subsequent filtrate with water to make 25 mL. Perform the test with this solution as the test solution, according to Method 1. Prepare the control solution with 1.5 mL of standard lead solution (not more than 30 ppm).

Loss on Drying Not more than 7.0 % (1 g, 150 ºC, 4 hours).

Assay Weigh accurately about 22 mg of Phenobarbital Sodium, add 15.0 mL of the internal standard solution and shake for 15 minutes using sonicator. Filter through a membrane filter with the pore size of not bigger than 0.5 μm and use the filtrate as the test solution. Separately, weigh accurately 20 mg of Phenobarbital RS, previously dried at 105 ºC for 2 hours, dissolve in 15.0 mL of the internal standard solution and use this solution as the standard solution. If necessary, mix and dissolve by sonication. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and calculate the ratios, Q_T and Q_S, of the peak area of phenobarbital to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of phenobarbital sodium } (C_{12}H_{11}N_2NaO_3) = \text{Amount (mg) of Phenobarbital RS} \times \frac{Q_T}{Q_S} \times 1.095$$

Internal standard solution—Dissolve 12.5 mg of caffeine in 100 mL of a mixture of pH 4.5 buffer solution and methanol (1 : 1).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Mobile phase: A mixture of pH 4.5 buffer solution and methanol (3 : 2).

Flow rate: 2 mL/minute.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution according to the above operating conditions, the resolution between their peaks of caffeine and phenobarbital is not less than 1.2 and the symmetry factor is not more than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution according to the above operating conditions, the relative standard deviation of the ratio of the peak area of phenobarbital to that of internal standard is not more than 2.0 %.

pH 4.5 buffer solution—To 6.6 g of sodium acetate trihydrate and 3.0 mL of acetic acid (100), add water to make 1000 mL. Adjust pH to 4.5 ± 0.1 with acetic acid (100), if necessary.

Containers and Storage ***Containers***—Tight containers.

Phenobarbital Sodium Tablets

Phenobarbital Sodium Tablets contain not less than 92.5 % and not more than 107.5 % of the labeled amount of phenobarbital sodium ($C_{12}H_{11}N_2NaO_3$: 254.22).

Method of Preparation Prepare as directed under Tablets, with Phenobarbital Sodium.

Identification (1) Dissolve 0.2 g of residue, obtained in the Assay, in 15 mL of diluted ethanol (1 in 4) in a water-bath, filter with glass crucible filter when it is hot and cool. Wash with small amount of diluted ethanol (1 in 4) ethanol, evaporate the filtrate in the cap-mounted test tube and dry the residue at 105 ºC for 1 hour: Determine the infrared spectra of the residue obtained above and Phenobarbital RS, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) A powder of Phenobarbital Sodium Tablets responds to the Qualitative Tests for sodium salt.

Disintegration Test It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Phenobarbital Sodium Tablets. Weigh accurately a portion of the powder, equivalent to 22 mg of phenobarbital sodium ($C_{12}H_{11}N_2NaO_3$), dissolve in 15 mL of internal standard solution, mix, and sonicate for 15 minutes. Filter, and use the filtrate as the test solution. Separately, weigh accurately about 20 mg of Phenobarbital RS (previously dried at 105 ºC for 2 hours), dissolve in 15 mL of internal standard solution, and use this solution as the standard solution. Proceed as directed in the Assay under Phenobarbital Sodium.

$$\text{Amount (mg) of phenobarbital sodium } (C_{12}H_{11}N_2NaO_3) = \text{Amount (mg) of Phenobarbital RS} \times \frac{Q_T}{Q_S} \times 1.095$$

Internal standard solution—Dissolve 12.5 mg of caffeine in 100 mL of a mixture of methanol and pH 4.5 buffer solution (1:1).

Containers and Storage ***Containers***—Tight containers.

Phenobarbital Tablets

Phenobarbital Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of phenobarbital ($C_{12}H_{12}N_2O_3$: 232.24).

Method of Preparation Prepare as directed under Tablets, with Phenobarbital.

Identification (1) Shake thoroughly a portion of powdered Phenobarbital Tablets, equivalent to 60 mg of phenobarbital according to the labeled amount, with 50 mL of chloroform and filter. Evaporate the filtrate to dryness and dry at 105 ºC for 2 hours. Determine the infrared spectra of the residue obtained above and Phenobarbital RS, respectively, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears in the absorption spectra, dissolve the residue and phenobarbital RS in chloroform, respectively, evaporate the ethyl acetate and repeat the test on the residues.

(2) The retention time of the principal peak in the chromatogram of the test solution corresponds to that of the standard solution, as obtained in the Assay.

Dissolution Test Perform the test with 1 tablet of Phenobarbital Tablets at 50 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of water as the dissolution solution. Take the dissolved solution after 45 minutes from start of the test and filter. Dilute the filtrate to a appropriate concentration, using alkaline borate buffer solution, pH 9.6

and use this solution as the test solution. Separately, weigh accurately a portion of Phenobarbital RS, previously dried at 105 ºC for 2 hours, dissolve in alkaline borate buffer solution, pH 9.6 to make same concentration with the test solution and use this solution as the standard solution. Determine the absorbances of the test solution and the standard solution at 240 nm as directed under Ultraviolet-visible Spectrophotometry.
The dissolution rate of Phenobarbital Tablets in 45 minutes is not less than 75 %.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Phenobarbital Tablets, weigh accurately a portion of the powder, equivalent to about 20 mg of phenobarbital ($C_{12}H_{12}N_2O_3$), add 15.0 mL of the internal standard solution, mix and shake for 15 minutes. Filter before use and use this filtrate as the test solution. Separately, weigh accurately 20 mg of Phenobarbital RS, previously dried at 105 ºC for 2 hours, dissolve in 15.0 mL of the internal standard solution and use this solution as the standard solution. If necessary, mix and dissolve by sonication. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and calculate the ratios, Q_T and Q_S, of the peak area of phenobarbital to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of phenobarbital } (C_{12}H_{12}N_2O_3) = \text{Amount (mg) of Phenobarbital RS} \times \frac{Q_T}{Q_S}$$

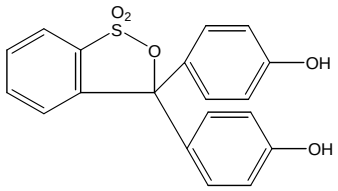
Internal standard solution—Dissolve 12.5 mg of caffeine in 100 mL of mixture of pH 4.5 buffer solution and methanol (1 : 1).

Operating conditions
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column, about 4 mm in internal diameter and about 25 cm in length, having octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).
Mobile phase: A mixture of pH 4.5 buffer solution and methanol (3 : 2).
Flow rate: 2 mL/minute.
System suitability
System performance: When the procedure is run with 10 μL of the standard solution according to the above operating conditions, the resolution between their peaks is not less than 1.2 and the symmetry factor is not more than 2.0.
System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of phenobarbital to that of the internal standard is not more than 2.0 %.

pH 4.5 buffer solution—To 6.6 g of sodium acetate trihydrate and 3.3 mL of acetic acid (100), add water to make 1000 mL. Adjust pH to 4.5 ± 0.1 with acetic acid (100), if necessary.

Containers and Storage ***Containers***—Tight containers.

Phenolsulfonphthalein



$C_{19}H_{14}O_5S$: 354.38

4-[3-(4-Hydroxyphenyl)-1,1-dioxo-2,1λ6-benzoxathiol-3-yl]phenol [*143-74-8*]

Phenolsulfonphthalein, when dried, contains not less than 98.0 % and not more than 101.0 % of phenolsulfonphthalein ($C_{19}H_{14}O_5S$).

Description Phenolsulfonphthalein appears as vivid red to dark red, crystalline powder.
Phenolsulfonphthalein is very slightly soluble in water or in ethanol (95).
Phenolsulfonphthalein dissolves in sodium hydroxide TS.

Identification (1) Dissolve 5 mg of Phenolsulfonphthalein in 2 to 3 drops of sodium hydroxide TS, add 2 mL of 0.05 mol/L bromine VS and 1 mL of dilute sulfuric acid, shake well and allow to stand for 5 minutes. Render the solution alkaline with sodium hydroxide TS: a deep blue-purple color develops.
(2) Dissolve 10 mg each of Phenolsulfonphthalein and Phenolsulfonphthalein RS in sodium carbonate solution (1 in 10) to make 200 mL. Take 5 mL of this solution and add sodium carbonate solution (1 in 10) to make 100 mL. Determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at same wavelengths.

Purity (1) ***Insoluble substances***—Take about 1.0 g of Phenolsulfonphthalein, accurately weighed, add 20 mL of a solution of sodium bicarbonate (1 in 40). Allow the mixture to stand for 1 hour with frequent shaking, add water to make 100 mL and allow to stand for 24 hours. Collect the insoluble substances using a tared glass filter (G4), wash with 25 mL of a solution of sodium bicarbonate (1 in 100) and wash with five 5 mL

volumes of water and dry at 105 ºC for 1 hour: the residue is not more than 0.2 %.

(2) ***Relative substances***—Dissolve 0.10 g of Phenolsulfonphthalein in 5 mL of dilute sodium hydroxide TS and use this solution as the test solution. Pipet 0.5 mL of this solution, add dilute sodium hydroxide TS to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL of each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of t-amyl alcohol, acetic acid (100) and water (4 : 1 : 1) to distance of about 15 cm and air-dry the plate. After leaving in ammonia vapor, examine under Ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot from the test solution are not more intense than the spot form the standard solution.

Loss on Drying Not more than 1.0 % (1 g, silica gel. 4 hours).

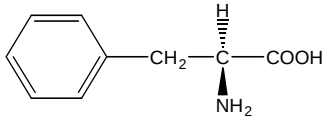
Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 0.15 g of Phenolsulfonphthalein, previously dried, transfer to an iodine flask, dissolve in 30 mL of a solution of sodium hydroxide (1 in 250) and add water to make exactly 200 mL. Add exactly measured 50 mL of 0.05 mol/L bromine VS, add 10 mL of hydrochloric acid to the solution quickly and stopper immediately. Allow the mixture to stand for 5 minutes with occasional shaking, add 7 mL of potassium iodide TS, stopper again immediately and shake gently for 1 minute. Titrate the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination and make any necessary correction.

Each mL of 0.05 mol/L bromine VS
= 4.430 mg of $C_{19}H_{14}O_5S$

Containers and Storage ***Containers***—Well-closed containers.

L-Phenylalanine



$C_9H_{11}NO_2$: 165.19

(2*S*)-2-Amino-3-phenylpropanoic acid [*63-91-2*]

L-Phenylalanine, when dried, contains not less than 98.5 % and not more than 101.0 % of L-phenylalanine ($C_9H_{11}NO_2$).

Description L-Phenylalanine appears as white crystals or crystalline powder, is odorless or has a faint, characteristic odor, and has a slightly bitter taste.
L-Phenylalanine is freely soluble in formic acid, sparingly soluble in water and practically insoluble in ethanol (95) or in ether.
L-Phenylalanine dissolves in dilute hydrochloric acid.

Identification Determine the infrared spectra of L-Phenylalanine and L-Phenylalanine RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

pH Dissolve 0.20 g of L-Phenylalanine in 20 mL of water by warming and cool: the pH of this solution is between 5.3 and 6.3.

Specific Optical Rotation $[\alpha]_D^{20}$: -33.0 ~ -35.5º (after drying, 0.5 g, water, 25 mL, 100 mm).

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of L-Phenylalanine in 10 mL of 1 mol/L hydrochloric acid: the solution is clear and colorless.

(2) ***Chloride***—Perform the test with 0.5 g of L-Phenylalanine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021 %).

(3) ***Sulfate***—Perform the test with 0.6 g of L-Phenylalanine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028 %).

(4) ***Ammonium***—Perform the test with 0.25 g of L-Phenylalanine. Prepare the control solution with 5.0 mL of standard ammonium solution (not more than 0.02 %).

(5) ***Heavy metals***—Dissolve 0.1 g of L-Phenylalanine in 40 mL of water and 2 mL of dilute acetic acid by warming, cool and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of standard lead solution, add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(6) ***Iron***—Weigh accurately 0.333 g of L-Phenylalanine, dissolve in water to make 45 mL, add 2 mL of hydrochloric acid, and use this solution as the test solution. To 1.0 mL of standard iron solution add water to make 45 mL, add 2 mL of hydrochloric acid, and use this solution as the standard solution. To the test solution and standard solution add 50 mg of ammonium peroxydisulfate and 3 mL of 30 % ammonium thiocyanate solution, and mix: the color obtained from the test solution is not more intense than that from the standard solution (not more than 30 ppm).

(7) ***Arsenic***—Dissolve 1.0 g of L-Phenylalanine in 3 mL of 1 mol/L hydrochloric acid TS and 2 mL of water by heating in a water-bath and perform the test

(not more than 2 ppm).

(8) ***Related substances***—Dissolve 0.10 g of L-Phenylalanine in 25 mL of water and use this solution as the test solution. Pipet 1 mL of the test solution and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3 : 1 : 1) to a distance of about 10 cm and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate and heat at 80 ºC for 5 minutes: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.3 % (1 g, 105 ºC, 3 hours).

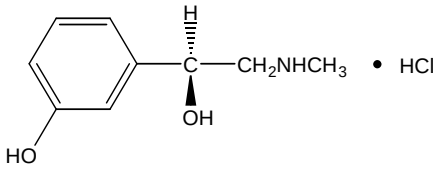
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.17 g of L-Phenylalanine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchioric acid VS
= 16.519 mg of $C_9H_{11}NO_2$

Containers and Storage ***Containers***—Tight containers.

Phenylephrine Hydrochloride



$C_9H_{13}NO_2 \cdot HCl$: 203.67

3-[(1*R*)-1-Hydroxy-2-(methylamino)ethyl]phenol hydrochloride [*61-76-7*]

Phenylephrine Hydrochloride, when dried, contains not less than 98.0 % and not more than 102.0 % of phenylephrine hydrochloride ($C_9H_{13}NO_2 \cdot HCl$).

Description Phenylephrine Hydrochloride appears as white crystals or crystalline powder, is odorless and has a bitter taste.

Phenylephrine Hydrochloride is very soluble in water, freely soluble in ethanol (95) and practically insoluble in ether.

pH—The pH of a solution of Phenylephrine Hydrochloride (1 in 100) is between 4.5 and 5.5.

Identification (1) Take 1 mL of a solution of Phenylephrine Hydrochloride (1 in 100), add 1 drop of copper (II) sulfate TS and 1 mL of a solution of sodium hydroxide (1 in 5): a blue color is observed. To this solution, add 1 mL of ether and shake vigorously: no blue color is observed in the ether layer.

(2) Take 1 mL of a solution of Phenylephrine Hydrochloride (1 in 100) and add 1 drop of iron (III) chloride TS: a persistent purple color is observed.

(3) Dissolve 0.3 g of Phenylephrine Hydrochloride in 3 mL of water, add 1 mL of ammonia TS and rub the inner side of the test tube with a glass rod: a precipitate is produced. Filter and collect the precipitate, wash with a few drops of ice-cold water and dry at 105 °C for 2 hours: it melts between 170 °C and 177 °C.

(4) A solution of Phenylephrine Hydrochloride (1 in 100) responds to the Qualitative Tests (2) for chloride.

Melting Point 140 ~ 145 °C

Specific Optical Rotation $[\alpha]_D^{20}$: -42.0 ~ -47.5° (after drying, 0.5 g, water, 10 mL, 100 mm).

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Phenylephrine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) ***Sulfate***—Take 0.5 g of Phenylephrine Hydrochloride and perform the test. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048 %).

(3) ***Ketone***—Dissolve 0.20 g of Phenylephrine Hydrochloride in 1 mL of water and add 2 drops of sodium nitroprusside TS, 1 mL of sodium hydroxide TS and then 0.6 mL of acetic acid (100): the solution has no more color than the following control solution.

Control solution—Prepare as directed above without Phenylephrine Hydrochloride.

(4) ***Related substances***—Weigh accurately 500 mg of Phenylephrine Hydrochloride, dissolve in methanol to make exactly 10 mL, and use this solution as the test solution. Separately, weigh accurately about 20 mg of Phenylephrine Hydrochloride RS, dissolve in methanol to make exactly 20 mL, and use this solution as the standard solution (1). Pipet 5 mL of the standard solution (1), add methanol to make exactly 10 mL, and use this solution as the standard solution (2). Pipet 1 mL of the standard solution (1), add methanol to make exactly 4 mL, and use this solution as the standard solution (3). Pipet 1 mL of the standard solution (1), add methanol to make exactly 10 mL, and use this solution as the

standard solution (4). Pipet 1 mL of the standard solution (1), add methanol to make exactly 20 mL, and use this solution as the standard solution (5). Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and standard solutions (2), (3), (4), and (5) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, and formic acid (7 : 2 : 1) to a distance of about 15 cm, and dry the plate in warm air. Spray a solution of anhydrous sodium carbonate (1 in 10), and a saturated solution of *p*-nitrobenzenediazonium fluoroborate, dry, and examine under ultraviolet light (main wavelength: 254 nm). The sum of the intensities of the spots other than the principal spot obtained from the test solution is not more intense than that from the standard solution (2) (not more than 1.0 %), and the intensity of each related substance is not more intense than that from the standard solution (3) (not more than 0.5 %).

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.2 % (1 g).

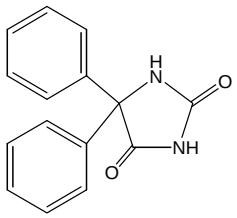
Assay Weigh accurately about 0.1 g of Phenylephrine Hydrochloride, previously dried, dissolve in 40 mL of water contained in an iodine flask, add exactly measured 50 mL of 0.05 mol/L bromine VS, then add 5 mL of hydrochloride acid and immediately stopper tightly. Shake the mixture and allow to stand for 15 minutes. To this solution, add 10 mL of potassium iodide TS carefully, stopper tightly immediately, shake thoroughly, allow to stand for 5 minutes and titrate with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination and make any necessary correction.

Each mL of 0.05 mol/L bromine VS
= 3.3945 mg of $C_9H_{13}NO_2 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Phenytoin



Diphenylhydantoin $C_{15}H_{12}N_2O_2$: 252.27

5,5-Diphenylimidazolidine-2,4-dione [*57-41-0*]

Phenytoin, when dried, contains not less than 99.0 % and not more than 101.0 % of phenytoin ($C_{15}H_{12}N_2O_2$).

Description Phenytoin is a white, crystalline powder or granule, is odorless and tasteless.

Phenytoin is sparingly soluble in ethanol (95) or in acetone, slightly soluble in ether and practically insoluble in water.

Phenytoin dissolves in sodium hydroxide TS.

Melting point—About 296 ºC (with decomposition).

Identification (1) Dissolve 20 mg of Phenytoin in 2 mL of ammonia TS and add 5 mL of silver nitrate TS: a white precipitate is produced.

(2) Boil a mixture of 10 mg of Phenytoin, 1 mL of ammonia TS and 1 mL of water and add drop-wise 2 mL of a mixture prepared from 50 mL of a solution of cupric sulfate (1 in 20) and 10 mL of ammonia TS: a red, crystalline precipitate is produced.

(3) Heat 0.1 g of Phenytoin with 0.2 g of sodium hydroxide and fuse: the gas evolved turns moistened red litmus paper blue.

(4) Add 3 mL of chlorinated lime TS to 0.1 g of Phenytoin, shake for 5 minutes and dissolve the oily precipitate in 15 mL of hot water. After cooling, add 1 mL of dilute hydrochloric acid drop-wise, then add 4 mL of water. Filter and collect the white precipitate thus obtained, wash with water and press it with dry filter paper to remove the accompanying water. Dissolve the precipitate with 1 mL of chloroform, add 5 mL of diluted ethanol (9 in 10) and rub the inner surface of the flask to produce a white, crystalline precipitate. Collect the precipitate, wash with ethanol (95) and dry: the melting point is between 165 ºC and 169 ºC.

Purity (1) ***Clarity and color of solution***—Dissolve 0.20 g of Phenytoin in 10 mL of 0.2 mol/L sodium hydroxide VS: the solution is clear and colorless. Then heat the solution: no turbidity is produced. Cool and mix the solution with 5 mL of acetone: the solution is clear and colorless.

(2) ***Acid or alkali***—Shake 2.0 g of Phenytoin with 40 mL of water for 1 minute, filter and perform the following tests using this filtrate as the test solution.

(i) To 10 mL of the test solution, add 2 drops of phenolphthalein TS: no color develops. Then add 0.15 mL of 0.01 mol/L sodium hydroxide VS: a red color develops.

(ii) To 10 mL of the test solution, add 0.30 mL of 0.01 mol/L hydrochloric acid VS and 5 drops of methyl red TS: a red to orange color develops.

(3) ***Chloride***—Dissolve 0.30 g of Phenytoin in 30 mL of acetone and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution from 0.60 mL of 0.01 mol/L hydrochloric acid VS, 30 mL of acetone and 6 mL of dilute nitric acid and add water to make 50 mL (not more than 0.071 %).

(4) ***Heavy metals***—Proceed with 1.0 g of Phenyto-

in according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(5) ***Related substances***—Weigh accurately about 0.1 g of Phenytoin, dissolve in methanol to make exactly 100 mL, and use this solution as the test solution. Separately, weigh accurately about 0.1 mg of Phenytoin RS, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions. Determine the peak areas of each solution by the automatic integration method, and calculate the amount of related substances by the following equation: the total amount of related substances other than benzophenone is not more than 0.9 %.

$$\text{Amount (\%) of related substances} = 100 \times \frac{C_S}{C_T} \times \frac{A_i}{A_S}$$

C_S: Concentration (μg/mL) of phenytoin in the standard solution

C_T: Concentration (μg/mL) of phenytoin in the test solution

A_i: Peak area of each related substance in the test solution

A_S: Peak area of phenytoin in the standard solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of methanol and water (55 : 45)

Flow rate: About 1.5 mL/minute

System suitability

System performance: Weigh accurately 1 mg of benzoin, dissolve in 100 mL of methanol, dissolve 10 mg of phenytoin in 10 mL of this solution, and use this solution as the system suitability solution. When the procedure is run with the system suitability solution under the above operating conditions, the relative retention times of phenytoin and benzoin are 0.75 and 1.0, respectively, with the resolution between these peaks being not less than 1.5.

(6) ***Benzophenone***—Weigh accurately about 0.1 g of Phenytoin, dissolve in methanol to make exactly 100 mL, and use this solution as the test solution. Separately, weigh accurately about 0.1 mg of benzophenone, dissolve in methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of benzophenone in the test solution and standard solution. Calculate the amount of benzophenone by the following equation: not more than 0.1 %.

$$\text{Amount (\%) of benzophenone} = 100 \times \frac{C_S}{C_T} \times \frac{A_T}{A_S}$$

C_S: Concentration (μg/mL) of benzophenone in the standard solution

C_T: Concentration (μg/mL) of phenytoin in the test solution

A_T: Peak area of benzophenone in the test solution

A_S: Peak area of benzophenone in the standard solution

Operating conditions

Proceed as directed in the operating conditions in the Purity (5) Related substances.

System suitability

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of benzophenone is not more than 5.0 %.

Loss on Drying Not more than 0.5 % (2 g, 105 ºC, 2 hours).

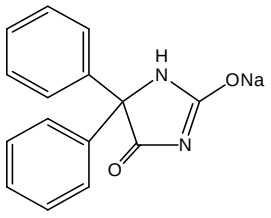
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.5 g of Phenytoin, previously dried, dissolve in 40 mL of ethanol (95) with the aid of gentle heating, add 0.5 mL of thymolphthalein TS immediately and titrate with 0.1 mol/L sodium hydroxide VS until a pale blue color develops. Then add 1 mL of pyridine, 5 drops of phenolphthalein TS and 25 mL of silver nitrate TS and titrate with 0.1 mol/L sodium hydroxide VS until a pale red color, which persists for 1 minute, develops.

Each mL of 0.1 mol/L sodium hydroxide VS
= 25.227 mg of $C_{15}H_{12}N_2O_2$.

Containers and Storage ***Containers***—Well-closed containers.

Phenytoin Sodium



Diphenylhydantoin Sodium $C_{15}H_{11}N_2NaO_2$: 274.25

Sodium 5,5-diphenylimidazolidin-3-ide-2,4-dione
[*630-93-3*]

Phenytoin Sodium contains not less than 98.0 % and not more than 102.0 % of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$), calculated on the dried basis.

Description Phenytoin Sodium appears as white crystals or crystalline powder, and is odorless.
Phenytoin Sodium is soluble in water or in ethanol (95) and practically insoluble in chloroform or in ether.

pH—The pH of a solution of Phenytoin Sodium (1 in 20) is about 12.
Phenytoin Sodium is hygroscopic.
A solution of Phenytoin Sodium absorbs carbon dioxide gradually when exposed to air and a crystalline precipitate of phenytoin is produced.

Identification (1) Put 0.3 g of Phenytoin Sodium in a separatory funnel, dissolve in 50 mL water and add 10 mL of dilute hydrochloric acid, extract with 100 mL of ether. Next extract 4 times with 25 mL of ether and combine the ether extracts and filter. Evaporate the filtrate on a water-bath to dryness and proceed with the residue as directed in the Identification under Phenytoin.

(2) Ignite 0.5 g of Phenytoin Sodium, cool and dissolve the residue in 10 mL of water: the solution changes red litmus paper to blue and responds to the Qualitative Tests (1) for sodium salt.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Phenytoin Sodium in 20 mL of freshly boiled and cooled water: the solution is clear and colorless. If any turbidity is produced, add 4.0 mL of 0.1 mol/L sodium hydroxide VS: the solution becomes clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Phenytoin Sodium according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Weigh accurately about 0.1 g of Phenytoin Sodium, dissolve in the mobile phase to make exactly 100 mL, and use this solution as the test solution. Separately, weigh accurately about 50 μg of benzophenone, about 0.1 mg of Phenytoin RS, about 0.9 mg of Phenytoin Related Substance I {diphenylgycine} RS, and about 0.9 mg of Phenytoin Related Sbustance II {diphenylhydantoic acid} RS, dissolve in the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of each solution by the automatic integration method, and calculate the amount of related substances by the following equation: the amounts of phenytoin related substance I and II are not more than 0.9 %, respectively, and the amount of benzophenone is not more than 0.1 %.

$$\text{Amount (\%) of related substances} = 100 \times \frac{C_S}{C_T} \times \frac{A_T}{A_S}$$

C_S: Concentration (μg/mL) of each related substance in the standard solution
C_T: Concentration (μg/mL) of Phenytoin Sodium in the test solution
A_S: Peak area of each related substance obtained from the standard solution
A_T: Peak area of each related substance obtained from the test solution

Calculate the amount of each other related substance by the following equation: the total amount of related substances other than benzophenone is not more than 0.9 %.

$$\text{Amount (\%) of each related substance} = 100 \times \frac{274.25}{252.27} \times \frac{C}{D} \times \frac{A_i}{A_S}$$

274.25: Molecular weight of phenytoin sodium
252.27: Molecular weight of phenytoin
C: Concentration (μgmL) of phenytoin in the standard solution
D: Concentration (μg/mL) of phenytoin sodium in the test solution
A_i: Peak area of related substances obtained from the test solution
A_S: Peak area of related substances obtained from the standard solution

Operating conditions
Detector: An ultraviolet absorption photometer (wavelength: 220 nm)
Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: An ordinary temperature
Mobile phase: A mixture of 0.05 mol/L ammonium dihydrogen phosphate buffer solution, adjusted to pH 2.5 with phosphoric acid, acetonitrile, and methanol (45 : 35 : 20)
Flow rate: About 1.5 mL/minute
System suitability
System performance: Dissolve about 75 mg of benzoin in 10 mL of methanol, and add a mixture of 0.05 mol/L ammonium dihydrogen phosphate buffer solution, adjusted to pH 2.5 with phosphoric acid, and acetonitrile (45 : 35) to make 50 mL. Pipet 1.0 mL of this solution, transfer to a 10 mL volumetric flask, add a solution prepared by dissolving 10.0 mg of Phenytoin RS in 100 mL of the mobile phase to volume, and use this solution as the system suitability solution. When the procedure is run with the system suitability solution

under the above operating conditions, the relative retention times of phenytoin and benzoin are 1.0 and 1.3, respectively, the resolution between these peaks is not less than 1.5, and the number of theoretical plates and symmetry factor of the peak of phenytoin are not less than 9400 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation is not more than 5.0 %.

Assay Weigh accurately about 25 mg of Phenytoin Sodium and dissolve in the mobile phase to make exactly 25 mL. Pipet 5 mL of this solution, add 10.0 mL of the internal standard solution, add the mobile phase to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately about 25 mg of Phenytoin RS, dissolve in the internal standard solution to make exactly 25 mL. Pipet 5 mL of this solution, add 10 mL of the internal standard solution, add the mobile phase to make exactly 50 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and calculate the ratios, Q_T and Q_S, of the peak area of phenytoin to that of the internal standard, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of phenytoin sodium } (C_{15}H_{12}N_2NaO_2) = \text{Amount (mg) of Phenytoin RS, calculated on the dried basis} \times \frac{Q_T}{Q_S} \times 1.087$$

Internal standard solution—A solution of butyl paraoxybenzoate in methanol (1 in 10000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and 15 cm to 30 cm in length, having octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Mobile phase: A mixture of 0.005 mol/L dibasic phosphate ammonium solution and methanol (50 : 50).

Flow rate: Adjust the retention time of phenytoin to be about 5 minutes.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution according to the above operating conditions, phenytoin and internal standard are eluted in this order with the resolution between their peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution according to the above operating conditions, the relative standard deviation of the ratios of the peak area of phenytoin to that of the internal standard is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Phenytoin Sodium for Injection

Diphenylhydantoin Sodium for Injection

$C_{15}H_{11}N_2NaO_2$: 274.25

Phenytoin Sodium for Injection is a preparation for injection which is reconstituted before use.

Phenytoin Sodium for Injection, when dried, contains not less than 98.5 % and not more than 101.0 % of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$) and contains not less than 92.5 % and not more than 107.5 % of the labeled amount of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$).

Method of Preparation Prepare as directed under Injections.

Description Phenytoin Sodium for Injection appears as white crystals or crystalline powder, and is odorless.

Phenytoin Sodium for Injection is soluble in water or in ethanol (95) and practically insoluble in chloroform or in ether.

pH—The pH of a solution of Phenytoin Sodium for Injection (1 in 20) is about 12.

Phenytoin Sodium for Injection is hygroscopic.

A solution of Phenytoin Sodium for Injection absorbs carbon dioxide gradually when exposed to air and a crystalline precipitate of phenytoin is produced.

Identification (1) Weigh a portion of Phenytoin Sodium for Injection, equivalent to 0.3 g of phenytoin according to the labeled amount, dissolve in 50 mL water and add 10 mL of dilute hydrochloric acid, extract with 100 mL of ether. Next extract 4 times with 25 mL of ether and combine the ether extracts and filter. Evaporate the filtrate on a water-bath to dryness and proceed with the residue as directed in the Identification under Phenytoin.

(2) Ignite 0.5 g of Phenytoin Sodium for Injection, cool and dissolve the residue in 10 mL of water: the solution changes red litmus paper to blue and responds to the Qualitative Tests (1) for sodium salt.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Phenytoin Sodium for Injection in 20 mL of freshly boiled and cooled water: the solution is clear and colorless. If any turbidity is produced, add 4.0 mL of 0.1 mol/L sodium hydroxide VS: the solution becomes clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Phenytoin Sodium for Injection according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

Loss on Drying Not more than 2.5 % (1 g, 105 ºC, 4 hours).

Sterility Test It meets the requirement.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately the mass of the content of not less than 10 samples of Phenytoin Sodium for Injection. Weigh accurately about 25 mg of Phenytoin Sodium of Injection and dissolve in the mobile phase to make exactly 25 mL. Pipet 5 mL of this solution, add 10.0 mL of the internal standard solution, add the mobile phase to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately about 25 mg of Phenytoin RS, dissolve in internal standard solution to make exactly 25 mL. Pipet 5 mL of this solution, add 10 mL of the internal standard solution, add the mobile phase to make exactly 50 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and calculate the ratios, Q_T and Q_S, of the peak area of phenytoin to that of the internal standard, for the test solution and the standard solution, respectively.

Amount (mg) of phenytoin sodium ($C_{15}H_{12}N_2NaO_2$)
= Amount (mg) of Phenytoin RS,
calculated on the dried basis $\times \frac{Q_T}{Q_S} \times 1.087$

Internal standard solution—A solution of butyl paraoxybenzoate in methanol (1 in 10000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and 15 cm to 30 cm in length, having octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Column temperature: An ordinary temperature.

Mobile phase: A mixture of 0.005 mol/L dibasic phosphate ammonium solution and methanol (50 : 50).

Flow rate: Adjust the retention time of phenytoin to be about 5 minutes.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution according to the above operating conditions, phenytoin and the internal standard are eluted in this order with the resolution between their peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution according to the above operating conditions, the relative standard deviation of the ratios of the peak area of phenytoin to that of the internal standard is not more than 2.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Phenytoin Tablets

Diphenylhydantoin Tablets

Phenytoin Tablets contain not less than 95.0 % and not more than 105.0 % of the labeled amount of phenytoin ($C_{15}H_{12}N_2O_2$: 252.27).

Method of Preparation Prepare as directed under Tablets, with Phenytoin.

Identification Weigh a portion of powdered Phenytoin Tablets, equivalent to about 0.3 g of Phenytoin, put it in a seperatory funnel with 1 mL of dilute hydrochloric acid and 10 mL of water. Extract with one 100 mL volume of ether, next four 25 mL volume of ether by shaking well, combine the extracts and filter. Evaporate the filtrate on a water-bath to dryness and perform the test with the residue as directed in the Identification under Phenytoin.

Dissolution Test Perform the test with 1 tablet of Phenytoin Tablets at 100 revolutions per minute according to Method 2, using 900 mL of 0.05 mol/L tris buffer solution as the dissolution solution. Take the dissolved solution 120 minutes after the start of the test, and filter. Discard the first 3 mL of the filtrate, pipet 10.0 mL of the subsequent filtrate, add the mobile phase to make exactly 50 mL, and use this solution as the test solution. Separately, weigh accurately a suitable amount of Phenytoin RS, dissolve in methanol so that each mL contains about 3.0 mg, and add the dissolution solution so that each mL contains about 0.06 mg. Pipet a suitable volume of this solution, add the mobile phase to make the same concentration as the test solution, and use this solution as the standard solution. Perform the test with 25 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak area of phenytoin in each solution. The dissolution rate of Phenytoin Tablets in 120 minutes is not less than 70 % (Q).

Dissolution rate (%) with respect to the labeled amount of phenytoin ($C_{15}H_{12}N_2O_2$)

$$= C_S \times \frac{A_T}{A_S} \times \frac{1}{C} \times 450000$$

C_S: Concentration (mg/mL) of the standard solution

C: Labeled amount (mg) of phenytoin ($C_{15}H_{12}N_2O_2$) in 1 tablet

0.05 mol/L Tris buffer solution—Dissolve 60.5 g of 2-amino-2-hydroxymethyl-1,3-propandiol in 6 L of water, add water to make 10 L, and adjust the pH to 9.0 ± 0.05 with phosphoric acid. Dissolve 100 g of sodium lauryl sulfate in 6 L of this solution, combine with the remaining solution, and mix well.

Triethylamine solution—To 1 mL of triethylamine add water to make 100 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (1.5 to 10 μm in particle diameter).

Mobile phase: A mixture of water, methanol, acetonitrile, triethylamine solution, and acetic acid (31) (500 : 270 : 230 : 5 : 1)

Flow rate: 1.5 mL/minute

System suitability

System performance: When the procedure is run with 25 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of phenytoin are not less than 6500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 5 times with 25 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of phenytoin is not more than 2.0 %.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Phenytoin Tablets. Weigh accurately a portion of the powder, equivalent to about 25 mg of phenytoin ($C_{15}H_{12}N_2O_2$), shake with the mobile phase for 10 minutes, add the mobile phase to make exactly 25 mL and filter. Discard the first 10 mL of the filtrate, take exactly 5 mL of the subsequent filtrate, add 10.0 mL of the internal standard solution, add the mobile phase to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately about 25 mg of Phenytoin RS (previously dry at 105 ºC for 2 hours and measure the loss on drying), dissolve in the internal standard solution to make exactly 25 mL. Pipet 5 mL of this solution, add 10.0 mL of the internal standard solution, add the mobile phase to make exactly 50 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and calculate the ratios, Q_T and Q_S, of the peak area of phenytoin to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of phenytoin } (C_{15}H_{12}N_2O_2) = \text{Amount (mg) of Phenytoin RS, calculated on the dried basis} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of butyl paraoxybenzoate in methanol (1 in 10,000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and 15 cm to 30 cm in length, having octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Column temperature: An ordinary temperature.

Mobile phase: A mixture of 0.005 mol/L ammonium hydrogen phosphate solution and methanol (50 : 50).

Flow rate: Adjust the retention time of phenytoin to be about 5 minutes.

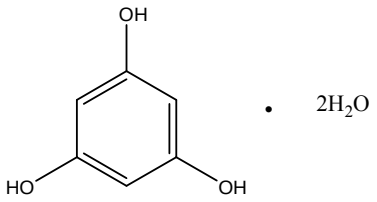
System suitability

System performance: When the procedure is run with 10 μL of the standard solution according to the above operating conditions, phenytoin and internal standard are eluted in this order with the resolution between their peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution according to the above operating conditions, the relative standard deviation of the ratios of the peak area of phenitoin to that of the internal standard is not more than 2.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Phloroglucinol Dihydrate



$C_6H_6O_3 \cdot 2H_2O$: 162.1

Benzene-1,3,5-triol dihydrate [*6099-90-7*]

Phloroglucinol Dihydrate, when dried, contains not less than 99.0 % and not more than 101.0 % of phloroglucinol ($C_6H_6O_3$: 126.1).

Description Phloroglucinol Dihydrate appears as white powder.

Phloroglucinol is freely soluble in ethanol (99.5) or in ethanol (95), sparingly soluble in water, and practically insoluble in dichloromethane.

Identification (1) Determine the infrared spectra of Phloroglucinol Dihydrate and Phloroglucinol RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Weigh accurately about 200 mg of Phloroglucinol Dihydrate, add methanol to make 10 mL, and use this solution as the test solution. Separately, weigh accurately 200 mg of Phloroglucinol RS, add methanol to make 10 mL, and use this solution as the standard solution. Spot 10 μL each of the test solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane, and anhydrous formic acid (125 : 75 : 4) to a distance of not less than 2/3 of the length of the plate, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the test solution corresponds in R_f value, position, and size to the principal spot from the standard solution.

pH Dissolve 2.5 g of Phloroglucinol Dihydrate in ethanol (95) to make 25 mL. To 10 mL of this solution add carbon dioxide-free water to make 100 mL: the pH of this solution is between 4.0 and 6.0.

Purity (1) ***Clarity and color of solution***—Dissolve 2.5 g of Phloroglucinol Dihydrate in ethanol (95) to make 25 mL: the solution is clear and has no more color than the control solution.

Control solution—To a mixture of 1.0 mL of cobalt (II) chloride hexahydrate stock CS, 2.4 mL of iron (III) chloride hexahydrate stock CS, and 0.4 mL of copper sulfate stock CS add 10 g/L hydrochloric acid to make 10 mL. To 12.5 mL of this solution add 10 g/L hydrochloric acid to make 100 mL.

(2) ***Chloride***—Perform the test with 0.5 g of Phloroglucinol Dihydrate. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid (not more than 0.02 %).

(3) ***Sulfate***—Perform the test with 0.5 g of Phloroglucinol Dihydrate. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid (not more than 0.05 %).

(4) ***Heavy metals***—Proceed with 1.0 g of Phloroglucinol Dihydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(5) ***Related substances***—Weigh accurately about 50 mg of Phloroglucinol Dihydrate, add the diluent to make 10 mL, and use this solution as the test solution. Pipet 1 mL of the test solution, and add the diluent to make 100 mL. Pipet 1 mL of this solution, add the diluent to make exactly 10 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of each solution by the automatic integration method, and calculate the amount of related substances in the test solution: phloroglucinol related substance I {pyrogallol}, phloroglucinol related substance II {phloroglucide}, benzene-1,2,4-triol, 2,6-dichlorophenol, phloroglucinol related substance III {4-chlororesorcinol}, and 3,5-dichloroaniline, having the relative retention times of about 0.9, about 1.3, about 0.7, about 1.8, about 1.5, and about 2.0 with respect to phloroglucinol, respectively, are not more than 0.15 %, respectively. Any other related substance is not more than 0.1 %, and the total amount of related substances is not more than 0.3 %. Exclude any related substance not more than 0.05 %. Use the peak areas of phloroglucinol related substance I, phloroglucinol related substance II, benzene-1,2,4-triol, 2,6-dichlorophenol, phloroglucinol related substance III, and 3,5-dichloroaniline after multiplying by their relative response factors, 0.6, 0.2, 0.7, 0.6, 0.6, and 0.4, respectively. Protect the test solution and standard solution from light after preparation, and use immediately.

Diluent—A mixture of the mobile phase A and mobile phase B (1 : 9)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 265 nm)

Column: A column about 4.0 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as direted in the following table.

Mobile phase A: Dissolve 1.36 g of potassium dihydrogen phosphate in 900 mL of water, adjust the pH to 3.0 with phosphoric acid, and add water to make 1000 mL.

Mobile phase B: Acetonitrile

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-9	100	0
9-15	100→50	0→50
15-25	50→20	50→80
25-30	20	80

Flow rate: 1.0 mL/minute (the elution time of phloroglucinol is about 12minutes)

System suitability

System performance: Dissolve 6 mg each of Phloroglucinol Related Substance I RS, resorcinol, and Phloroglucinol Related Substance II RS in the diluent

to make 10 mL, add 2 mL of the test solution, and add the diluent to make 20 mL. Pipet 1 mL of this solution, add the diluent to make 50 mL, and use this solution as the system suitability solution. When the procedure is run with 10 μL of the system suitability solution under the above operating conditions, the resolution between the peaks of phloroglucinol related substance I and phloroglucinol related substance II is not less than 2.5, and the resolution between the peaks of phloroglucinol related substance II and resorcinol is not less than 4.0.

Loss on Drying 20.0 ~ 23.0 % (1 g, 105 °C).

Residue on Ignition Not more than 0.1 % (1 g).

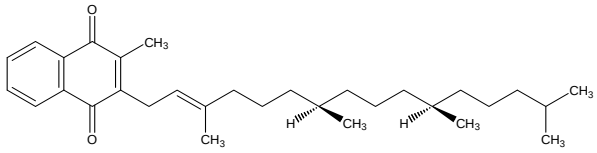
Assay Weigh accurately about 0.6 g of Phloroglucinol Dihydrate, and dissolve in 50 mL of water. Titrate this solution with 1 mol/L sodium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry).

Each mL of 1 mol/L sodium hydroxide VS
= 63.05 mg of $C_6H_6O_3$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Phytonadione



Phytomenadione
Vitamin K_1 $C_{31}H_{46}O_2$: 450.70

2-Methyl-3-((7*R*,11*R*,*E*)-3,7,11,15-tetramethylhexadec-2-en-1-yl)naphthalene-1,4-dione
[*84-80-0*]

Phytonadione contains not less than 97.0 % and not more than 102.0 % of phytonadione ($C_{31}H_{46}O_2$).

Description Phytonadione is a clear yellow to orange-yellow, viscous liquid and is odorless.
Phytonadione is miscible with isooctane.
Phytonadione is soluble in ethanol (99.5) and practically insoluble in water.
Phytonadione decomposes gradually and changes to red-brown by light.

Specific gravity— d_{20}^{20} : About 0.967.

Identification (1) Determine the absorption spectra of solutions of Phytonadione and Phytonadione RS in isooctane (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths. Seperately, determine the absorption spectra of solutions of Phytonadione and Phytonadione RS in isooctane (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Phytonadione and Phytonadione RS as directed in the potassium bromide disk method under Infrared Spectrophotometry, and compare the spectrum with the standard spectrum: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20} : 1.525 ~ 1.529.

Purity (1) ***Ratio of absorbances*** —Determine the absorbances, A_1, A_2 and A_3 of a solution of Phytonadione in isooctane (1 in 100000) at 248.5 nm, 253.5 nm and 269.5 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry: the ratio A_2/A_1 is between 0.69 and 0.73, and the ratio A_2/A_3 is between 0.74 and 0.78. Determine the absorbances, A_4 and A_5 of a solution of Phytonadione in isooctane (1 in 10000) at 284.5 nm and 326 nm, respectively: the ratio A_4/A_5 is between 0.28 and 0.34.

(2) ***Heavy metals***—Carbonize 1.0 g of Phytonadione by gentle heating. Cool, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and ignite the ethanol to burn. Cool, add 1 mL of sulfuric acid, proceed according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Menadione***—Dissolve 20 mg of Phytonadione in 0.5 mL of a mixture of water and ethanol (95) (1 : 1), add 1 drop of a solution of 3-methyl-1-phenyl-5-pyrazolone in ethanol (95) (1 in 20) and 1 drop of ammonia solution (28), and allow to stand for 2 hours: no blue-purple color develops.

Isomer ratio Perform this procedure rapidly without exposure to light. Dissolve 30 mg of Phytonadione in 50 mL of the mobile phase. To 4 mL of this solution add the mobile phase to make 25 mL. To 10 mL of this solution add the mobile phase to make 25 mL, and use this solution as the test solution. Perform the test with 50 μL of the test solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas of *Z*-isomer and *E*-isomer, A_{TZ} and A_{TE}: $A_{TZ}/(A_{TZ}+A_{TE})$ is between 0.05 and 0.18.

Operating conditions

Proceed as directed in the operating conditions in the Assay.

System suitability

System performance: When the procedure is run with 50 μL of the standard solution according to the above operating conditions, *Z*-isomer and *E*-isomer are eluted in this order with the resolution between then

being not less than 1.5.

System repeatability: When the test is repeated 6 times with 50 μL each of the standard solution according to the above operating conditions, the relative standard deviation of the sum of peak areas of *E*-isomer peak and *Z*-isomer peak is not more than 1.0 %.

Assay Perform this procedure rapidly without exposure to light. Weigh accurately about 30 mg each of Phytonadione and Phytonadione RS, and dissolve each in the mobile phase to make exactly 50 mL. Pipet 4 mL each of these solutions, and add the mobile phase to make exactly 25 mL. To exactly 10 mL each of these solutions add exactly 7 mL of the internal standard solution and the mobile phase to make exactly 25 mL, and use these as the test solution and standard solution, respectively. Perform the test with 50 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the total area of the peaks of *Z*-isomer and *E*-isomer to the peak area of the internal standard in each solution.

$$\text{Amount (mg) of phytonadione } (C_{31}H_{46}O_2) = W_S \times \frac{Q_T}{Q_S}$$

Internal standard solution—The mobile phase solution (1 in 400) of cholesterol benzoic acid.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquidchromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30 °C.

Mobile phase: A mixture of hexane and *n*-amyl alcohol (4000 : 3).

Flow rate: Adjust the flow rate so that the retention time of the lately eluted peak of two peaks of phytonadione is about 25 minutes.

System suitability

System performance: When the procedure is run with 50 μL of the standard solution according to the above operating conditions, the internal standard, *Z*-isomer and *E*-isomer are eluted in this order with the resolution of *Z*-isomer and *E*-isomer being not less than 1.5.

System repeatability: When the test is repeated 6 times with 50 μL each of the standard solution according to the above operating conditions, the relative standard deviation of the ratio of the sum of peak areas of *E*-isomer and *Z*-isomer to the peak area of internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and in a cold place.

Phytonadione Injection

Vitamin K_1 Injection

Phytonadione Injection is an aqueous solution for injection. Phytonadione Injection contains not less than 90.0 % and not more than 110.0 % of the labeled amount of phytonadione ($C_{31}H_{46}O_2$: 450.70).

Method of Preparation Prepare as directed under Injections, with Phytonadione. Phytonadione Injection contains suitable solubilizing or dispersing agents.

Identification The retention time of principal peak in the chromatogram of the Assay preparation corresponds to that of the standard preparation, as obtained in the assay.

pH 3.5 ~ 7.0.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 14.0 EU/mg of phytonadione.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay Pipet a volume of Phytonadione Injection, equivalent to 10 mg of phytonadione ($C_{31}H_{46}O_2$) according to the labeled amount, add mobile phase to make exactly 10 mL and mix. Pipet 1.0 mL of this solution, dilute with mobile phase to make exactly 10 mL and use this solution as the test solution. And in case of Phytonadione Injection containing less than 10 mg Phytonadione per mL, pipet accurately a volume of Phytonadione Injection, equivalent to 1 mg of phytonadione ($C_{31}H_{46}O_2$), add mobile phase to make exactly 10 mL. Separately, weigh accurately about 10 mg of Phytonadione RS, and dissolve in the mobile phase to make exactly 10 mL. Pipet 1.0 mL of this solution, dilute with mobile phase to make exactly 10 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the peak areas, A_T and A_S, of phytonadione for the test solution and the standard solution, respectively.

Amount (mg/mL) of phytonadione ($C_{31}H_{46}O_2$)

$$= D \times \frac{C}{V} \times \frac{A_T}{A_S}$$

D: 100 (in case of Phytonadione Injection containing 10 mg or more of phytonadione per mL) or 10 (in case of Phytonadione Injection containing less than 10 mg of phytonadione per mL).

C: Concentration (mg/mL) of Phytonadione in the standard solution

V: Volume (mL) of Phytonadione Injection taken.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm to 10 μm in particle diameter).

Mobile phase: A mixture of ethanol (99.5) and water (95 : 5).

Flow rate: 0.7 mL/minute.

System suitability

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution according to the above operating conditions, the relative standard deviation of the peak areas of phytonadione is not more than 1.5 %.

Containers and Storage ***Containers***—Hermetic containers.

Storage—Light-resistant.

Phytonadione Tablets

Vitamin K_1 Tablets

Phytonadione Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of phytonadione ($C_{31}H_{46}O_2$: 450.70).

Method of Preparation Prepare as directed under Tablets, with Phytonadione.

Identification (1) Dissolve a portion of the powdered Phytonadione Tablets, equivalent to about 10 mg of Phytonadione according to the labeled amount, in 750 mL of ethanol (99.5) with shaking, add ethanol (99.5) to make 1000 mL, mix and filter, use the filtrate as the test solution. Separately, proceed the same manner used in the test solution with Phytonadione RS, use this solution as the standard solution. Perform the test with test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelenghs.

(2) The retention time of the principal peak from the test solution in the Assay is the same with the retention time of peak from the standard solution..

Disintegration Test It meets the requirement (disintegration time is 30 minutes).

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Phytonadione Tablets. Weigh accurately a portion of the powder, equivalent to about 5 mg of phytonadione ($C_{31}H_{46}O_2$), add 20 mL of dehydrated ethanol, dissolve with vigorous shaking for 15 minutes and add ethanol (99.5) to make exactly 50 mL. Filter this solution and use this filtrate as the test solution. Separately, weigh accurately about 5 mg of Phytonadione RS, dissolve in ethanol (99.5) to make exactly 50 mL, use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions: determine the main peak areas, A_T and A_S, of Phytonadione for the test solution and the standard solution, respectively.

Amount (mg) of phytonadione ($C_{31}H_{46}O_2$)

$$= \text{Amount (mg) of Phytonadione RS (mg)} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm to 10 μm in particle diameter).

Mobile phase: A mixture of ethanol (99.5) and water (95 : 5).

Flow rate: 1.5 mL/minute.

System suitability

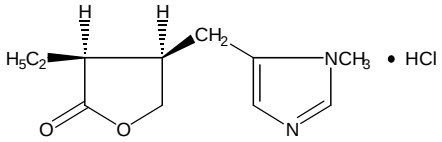
System performance: When the procedure is run with 10 μL of the standard solution according to the above operating conditions, a symmetry factor is not more than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution according to the above operating conditions, the relative standard deviation of the peak areas of phytonadione is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Pilocarpine Hydrochloride



$C_{11}H_{16}N_2O_2 \cdot HCl$: 244.72

(3*S*,4*R*)-3-Ethyl-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]dihydrofuran-2(3*H*)-one [*54-71-7*]

Pilocarpine Hydrochloride, when dreid, contains not less than 99.0 % and not more than 101.0 % of pilocarpine hydrochloride ($C_{11}H_{16}N_2O_2 \cdot HCl$).

Description Pilocarpine Hydrochloride appears as colorless crystals or white powder, is odorless and has a slightly bitter taste.
Pilocarpine Hydrochloride is very soluble in acetic acid (100), freely soluble in water, in methanol or in ethanol (95), soluble in acetic anhydride and practically insoluble in ether.

pH—The pH of a solution of Pilocarpine Hydrochloride (1 in 10) is between 3.5 and 4.5.
Pilocarpine Hydrochloride is hygroscopic.
Pilocarpine Hydrochloride is affected by light.

Identification (1) Dissolve 0.1 g of Pilocarpine Hydrochloride in 5 mL of water, add 1 drop of dilute nitric acid, 1 mL of hydrogen peroxide TS, 1 mL of chloroform and 1 drop of a potassium dichromate solution (1 in 300) and shake the mixture vigorously: a purple color is observed in the chloroform layer, no color or a pale yellow color is observed in the aqueous layer.

(2) Take 1 mL of a solution of Pilocarpine Hydrochloride (1 in 20), add 1 mL of dilute nitric acid and 2 to 3 drops of silver nitrate TS: a white precipitate or opalescence is produced.

Melting Point 200 ~ 203 °C.

Purity (1) ***Sulfate***—Dissolve 0.5 g of Pilocarpine Hydrochloride in 20 mL of water and use this solution as the test solution. To 5.0 mL of the test solution, add 1 mL of dilute hydrochloric acid and 0.5 mL of barium chloride TS: no turbidity is produced.

(2) ***Nitrate***—To 2.0 mL of the test solution obtained in (1), add 2 mL of iron (II) sulfate TS and superimpose the mixture upon 4 mL of sulfuric acid: no dark brown color is observed at the zone of contact.

(3) ***Related substances***—Dissolve 0.3 g of Pilocarpine Hydrochloride in 10 mL of methanol and use this solution as the test solution. Pipet 1 mL of the test solution, add methanol to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia water (85 : 14 : 2) to a distance of about 13 cm and dry the plate at 105 °C for 10 minutes. Cool and spray evenly bismuth potassium iodide TS on the plate: the spots other than the principal spot from the test solution are not more intense than the spot obtained from the standard solution.

(4) ***Readily carbonizable substances***—Take 0.25 g of Pilocarpine Hydrochloride and perform the test: the solution has no more color than Color Matching Fluid B.

Loss on Drying Not more than 3.0 % (1 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.5 % (0.1 g).

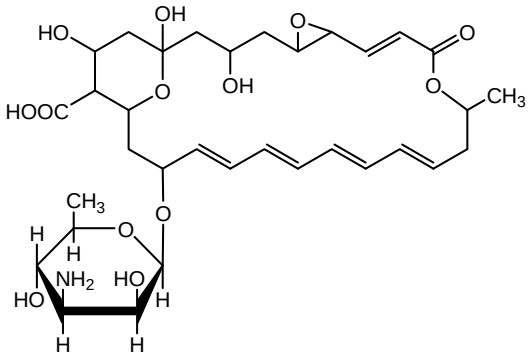
Assay Weigh accurately about 0.5 g of Pilocarpine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.1 mol/L perchloric acid VS (potentiometic titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 24.472 mg of $C_{11}H_{16}N_2O_2 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Pimaricin



$C_{33}H_{47}NO_{13}$: 665.73

(1*R*,3*S*,5*R*,7*R*,8*E*,12*R*,14*E*,16*E*,18*E*,20*E*,22*R*,24*S*,25*R*,26*S*)-22-[(3-Amino-3,6-dideoxy-β-D-mannopyranosyl)oxy]-1,3,26-trihydroxy-12-methyl-10-oxo-6,11,28-Trioxatricyclo[22.3.1.$0^{5,7}$]octacosa-8,14,16,18,20-pentaene-25-carboxylic acid [*7681-93-8*]

Pimaricin is a polyene macrolide substance having antifungal activity produced by the growth of *Streptomyces natalensis*.

Pimaricin contains not less than 900 μg (potency) and not more than 1020 μg (potency) per mg of pimaricin ($C_{33}H_{47}NO_{13}$: 665.73), calculated on the anhydrous basis.

Description Pimaricin appears as white to yellowish white crystalline powder.
Pimaricin is slightly soluble in methanol or in acetic acid (100), and practically insoluble in water or in ethanol (99.5).

Identification (1) To 3 mg of Pimaricin add 1 mL of hydrochloric acid, and shake: a blue-purple color develops.

(2) Dissolve separately 5 mg each of Pimaricin and Pimaricin RS in a solution of acetic acid (100) in methanol (1 in 100) to make 1000 mL. Determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Specific Optical Rotation $[\alpha]_D^{20}$: +243 ~ +259° (0.1 g, acetic acid (100), 25 mL, 100 mm).

pH Suspend 0.1 g (potency) of Pimaricin in 10 mL of water: the pH of this suspension is between 4.0 and 7.0.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Pimaricin according to Method 4, and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

(2) ***Related substances***—Dissolve 20 mg of Pimaricin in methanol to make 100 mL, and use this solution as the test solution. Perform the test with 10 μL of the test solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method. Calculate the sum of the amounts of substances other than pimaricin by the area percentage method: not more than 4.0 %.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 303 nm)

Column: A stainless steel column about 3.9 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: Dissolve 1.0 g of ammonium acetate in 1000 mL of a mixture of water, methanol, and tetrahydrofuran (47 : 44 : 2).

Flow rate: Adjust the flow rate so that the retention time of pimaricin is about 10 minutes.

System suitability

Test for required detectability: Pipet 1 mL of the test solution, add methanol to make exactly 100 mL, and use this solution as the system suitability solution. Pipet 1 mL of the system suitability solution, and add methanol to make exactly 10 mL. Confirm that the peak area of pimaricin obtained from 10 μL of this solution is equivalent to 7 to 13 % of that from the system suitability solution.

System performance: When the procedure is run with 10 μL of the system suitability solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of pimaricin are not less than 1500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the peak area of pimaricin is not more than 2.0 %.

Time span of measurement: About 3 times as long as the retention time of pimaricin.

Water 6.0 ~ 9.0 % (0.2 g, volumetric titration, direct titration).

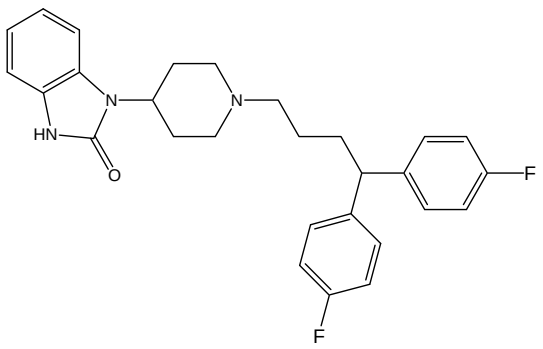
Assay Weigh accurately about 25 mg each of Pimaricin and Pimaricin RS, and dissolve each in methanol to make exactly 100 mL. To 2 mL each of these solutions add a solution of acetic acid (100) in methanol (1 in 100) to make exactly 100 mL, and use these solutions as the test solution and standard solution, respectively. Determine the absorbances at 295.5 nm, A_{T1} and A_{S1}, at 303 nm, A_{T2} and A_{S2}, and at 311 nm, A_{T3} and A_{S3}, of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount [μg (potency)] of pimaricin } (C_{33}H_{47}NO_{13}) = \text{Amount [μg (potency)] of Pimaricin RS} \times \frac{A_{T2} - \dfrac{A_{T1} + A_{T3}}{2}}{A_{S2} - \dfrac{A_{S1} + A_{S3}}{2}}$$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Pimozide



$C_{28}H_{29}F_2N_3O$: 461.55

1-{1-[4,4-Bis(4-fluorophenyl)butyl]piperidin-4-yl}-1*H*-benzo[d]imidazol-2(3*H*)-one [*2062-78-4*]

Pimozide contains not less than 98.0 % and not more than 102.0 % of pimozide ($C_{28}H_{29}F_2N_3O$), calculated on the dried basis.

Description Pimozide is a white, crystalline powder, is odorless, and has bitter taste.
Pimozide is freely soluble in acetic acid (100), slightly soluble in methanol or in ethanol (99.5), and practically insoluble in water.

Identification (1) Dissolve 35 mg each of Pimozide and Pimozide RS in 0.1 mol/L hydrochloric acid methanol solution (1 in 10) to make 100 mL, pipet 5.0 mL of this solution and add 0.1 mol/L hydrochloric acid methanol solution (1 in 10) to make 50 mL. Determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Pimozide and Pimozide RS, as directed in the potassium bromide disk method under lnfrared Spectrophotornetry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 216 ~ 220 °C.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Pimozide according to Method 2 under Heavy Metals Limit Test, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Arsenic***—Prepare the test solution with 1.0 g of Pimozide according to Method 3, and perform the test (not more than 2 ppm).

(3) ***Related substances***—Weigh accurately about 0.10 g of Pimozide, dissolve in exactly 10 mL of methanol, and use this solution as the test solution. Pipet 1 mL of this solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method: the area of any peak other than pimozide from the test solution is not larger than the peak area of pimozide from the standard solution, and the total area of the peaks other than pimozide from the test solution is not larger than 1.5 times the peak area of pimozide from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: Dissolve 2.5 g of ammonium acetate and 8.5 g of tetrabutylammonium hydrogen sulfate in water to make 1000 mL.

Mobile phase B: Acetonitrile

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-10	80→70	20→30
10-15	70	30

Flow rate: 2.0 mL/minute

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 10 mL. Confirm that the peak area of pimozide obtained from 10 μL of this solution is equivalent to 8 to 12 % of that from the standard solution.

System performance: Dissolve 5 mg of Pimozide and 2 mg of mebendazole in methanol to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, mebendazole and pimozide are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pimozide is not more than 2.0 %.

Time span of measurement: About 1.5 times as long as the retention time of pimozide.

Loss on Drying Not more than 0.5 % (0.5 g, in vacuum, 80 °C, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

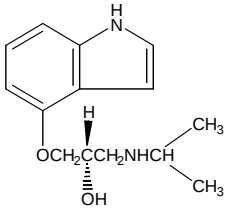
Assay Weigh accurately about 0.5 g of Pimozide, dissolve in 40 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 46.155 mg of $C_{28}H_{29}F_2N_3O$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Pindolol



and enantiomer

$C_{14}H_{20}N_2O_2$: 248.32

1-[(1*H*-indol-4-yl)oxy]-3-(isopropylamino)propan-2-ol
[*13523-86-9*]

Pindolol, when dried, contains not less than 98.5 % and not more than 101.0 % of pindolol ($C_{14}H_{20}N_2O_2$).

Description Pindolol is a white, crystalline powder and has a slight, characteristic odor.
Pindolol is sparingly soluble in methanol, slightly soluble in ethanol (95) and practically insoluble in water or in ether.
Pindolol dissolves in dilute sulfuric acid or in acetic acid (100).

Identification (1) Take 1 mL of a solution of Pindolol in methanol (1 in 10000), add 1 mL of a solution of 1-(4-pyridyl)-pyridinium chloride hydrochloride (1 in 1000) and 1 mL of sodium hydroxide TS, then add 1 mL of hydrochloric acid: a blue to blue-purple color, changing to red-purple, develops.

(2) Dissolve 50 mg of Pindolol in 1 mL of dilute sulfuric acid and add 1 mL of Reinecke salt TS: a pale red precipitate is produced.

(3) Determine the absorption spectra of solutions of Pindolol and Pindolol RS in methanol (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared spectra of Pindolol and Pindolol RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting Point 169 ~ 173 ºC.

Absorbance $E_{1\,cm}^{1\%}$ (264 nm): 333 ~ 350 (10 mg, methanol, 500 mL).

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Pindolol in 10 mL of acetic acid (100) and observe immediately: the solution is clear and has no more color than the following control solution.

Control solution—Pipet 4 mL of Color Matching Fluid A, add exactly 6 mL of water and mix.

(2) ***Heavy metals***—Proceed with 1.0 g of Pindolol according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Pindolol according to Method 3 and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 0.10 g of Pindolol in 10 mL of methanol and use this solution as the test solution. Pipet 2 mL of the test solution and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 20 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and isopropylamine (5 : 4 : 1) to a distance of about 12 cm and air-dry the plate. Spray evenly diluted sulfuric acid (3 in 5) and a sodium nitrite solution (1 in 50) on the plate: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

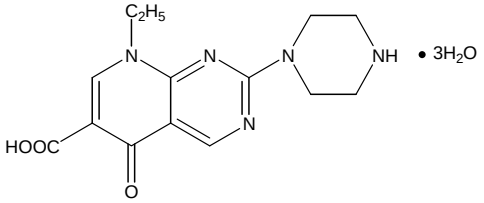
Assay Weigh accurately about 0.5 g of Pindolol, previously dried, dissolve in 80 mL of methanol and titrate with 0.1 mol/L hydrochloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L hydrochloric acid VS
= 24.832 mg of $C_{14}H_{20}N_2O_2$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Pipemidic Acid Hydrate



$C_{14}H_{17}N_5O_3 \cdot 3H_2O$: 357.36

8-Ethyl-5-oxo-2-(piperazin-1-yl)-5,8-dihydropyrido[2,3-d]pyrimidine-6-carboxylic acid
[*72571-82-5*]

Pipemidic Acid Hydrate contains not less than 98.5 % and not more than 101.0 % of pipemidic acid ($C_{14}H_{17}N_5O_3$: 303.32), calculated on the anhydrous basis.

Description Pipemidic Acid Hydrate appears as pale yellow crystalline powder.
Pipemidic Acid Hydrate is freely soluble in acetic acid (100), very slightly soluble in water or in ethanol (99.5) and practically insoluble in methanol.
Pipemidic Acid Hydrate dissolves in sodium hydroxide TS.
Pipemidic Acid Hydrate is gradually colored by light.

Melting point—About 250 ºC (with decomposition).

Identification (1) Dissolve 0.1 g of Pipemidic Acid Hydrate in 20 mL of sodium hydroxide TS, heat under a reflux condenser in a water-bath for 1 hour and cool. To 2 mL of this solution, add 1 drop of phenolphthalein TS, neutralize with dilute acetic acid, add 1 mL of dilute acetic acid, then add 4 mL of a solution of *p*-benzoquinone in methanol (1 in 1000) and boil gently: an orange color develops.

(2) Dissolve 0.1 g each of Pipemidic Acid Hydrate and Pipemidic Acid Hydrate RS in 20 mL of sodium hydroxide TS and add water to make 200 mL. To 1 mL each of these solutions, add water to make 100 mL and determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Pipemidic Acid Hydrate and Pipemidic Acid Hydrate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) ***Chloride***—Dissolve 1.0 g of Pipemidic Acid Hydrate in 35 mL of water and 10 mL of sodium hydroxide TS, shake well with 15 mL of dilute nitric acid and filter through a glass filter (G3). To 30 mL of the filtrate, add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test. Prepare the control solution as follows: to 0.30 mL of 0.01 mol/L hydrochloric acid VS, add 5 mL of sodium hydroxide TS, 13.5 mL of dilute nitric acid and water to make 50 mL (not more than 0.021 %).

(2) ***Sulfate***—Dissolve 1.0 g of Pipemidic Acid Hydrate in 35 mL of water and 10 mL of sodium hydroxide TS, shake well with 15 mL of dilute hydrochloric acid and filter through a glass filter (G3). To 30 mL of the filtrate, add water to make 50 mL. Perform the test. Prepare the control solution as follows: to 0.50 mL of 0.005 mol/L sulfuric acid VS, add 5 mL of sodium hydroxide TS, 7.5 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048 %).

(3) ***Heavy metals***—Proceed with 2.0 g of Pipemidic Acid Hydrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(4) ***Arsenic***—Prepare the test solution with 1.0 g of Pipemidic Acid Hydrate according to Method 3 and perform the test (not more than 2 ppm).

(5) ***Related substances***—Dissolve 0.10 g of Pipemidic Acid Hydrate in 10 mL of diluted acetic acid (100) (1 in 20) and use this solution as the test solution. Pipet 1.0 mL of the test solution, add diluted acetic acid (100) (1 in 20) to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, formic acid and triethylamine (25 : 15 : 5 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Water 14.5 ~ 16.0 % (20 mg, volumetric titration).

Residue on Ignition Not more than 0.1 % (1 g).

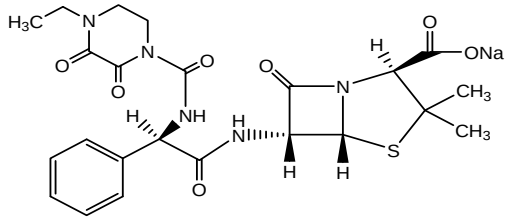
Assay Weigh accurately about 0.35 g of Pipemidic Acid Hydrate, previously dried, dissolve in 40 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 30.33 mg of $C_{14}H_{17}N_5O_3$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Piperacillin Sodium



$C_{23}H_{26}N_5NaO_7S$: 539.54

Sodium (2*S*,5*R*,6*R*)-6-{[(2*R*)-2-[(4-ethyl-2,3-dioxopiperazine-1-carbonyl)amino]-2-phenyl-acetyl]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [*59703-84-3*]

Piperacillin Sodium contains not less than 863 μg (po-

tency) per mg of piperacillin ($C_{23}H_{27}N_5O_7S$: 517.56), calculated on the anhydrous basis.

Description Piperacillin Sodium appears as white powder or masses.
Piperacillin Sodium is very soluble in water, freely soluble in methanol or in ethanol (95), and practically insoluble in acetonitrile.

Identification (1) Determine the infrared spectra of Piperacillin Sodium and Piperacillin Sodium RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Piperacillin Sodium responds to the Qualitative Tests (1) for sodium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: +175 ~ +190° (0.8 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g of Piperacillin Sodium in 4 mL of water is between 5.0 and 7.0.

Purity (1) ***Clarity or color of solution***—Dissolve 1.0 g of Piperacillin Sodium in 10 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***— Proceed with 2.0 g of Piperacillin Sodium according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***— Prepare the test solution with 2.0 g of Piperacillin Sodium according to Method 4 and perform the test (not more than 1 ppm).

(4) ***Related substances***—Weigh accurately about 0.1 g of Piperacillin Sodium, dissolve in the mobile phase A to make exactly 50 mL, and use this solution as the test solution. Pipet 1 mL of this solution, add the mobile phase A to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method: the peak area of ampicillin from the test solution, having the retention time of about 7 minutes, is not larger than 1/2 times the peak area of piperacillin from the standard solution, the sum of the peak areas of related substance I, having the retention times of about 17 minutes and about 21 minutes, is not larger than 2 times the peak area of piperacillin from the standard solution, the peak area of related substance II, having the retention time of about 56 minutes, is not larger than the peak area of piperacillin from the standard solution, and the total area of the peaks other than piperacillin is not larger than 5 times the peak area of piperacillin from the standard solution. Use the peak areas of ampicillin, related substance I, and related substance II after multiplying by their relative response factors, 1.39, 1.32, and 1.11, respectively.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: A mixture of water, acetonitrile, and 0.2 mol/L potassium dihydrogen phosphate TS (45 : 4 : 1)

Mobile phase B: A mixture of acetonitrile, water, and 0.2 mol/L potassium dihydrogen phosphate (25 : 24 : 1)

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-7	100	0
7-13	100→83	0→17
13-41	83	17
14-56	83→20	17→80
56-60	20	80

Flow rate: 1.0 mL/minute. The retention time of piperacillin is about 33 minutes under this condition.

Time span of measurement: About 1.8 times as long as the retention time of piperacillin, beginning after the solvent peak.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of piperacillin are not less than 15000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 3 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of piperacillin is not more than 2.0 %.

(5) ***Dimethylaniline***—Weigh accurately about 1.0 g of Piperacillin Sodium, add 5 mL of 1 mol/L sodium hydroxide TS and 1 mL of the internal standard solution, centrifuge if necessary, and use the supernatant liquid as the test solution. Separately, weigh accurately about 50 mg of dimethylaniline, and add 2.0 mL of hydrochloric acid and water to make 50 mL. Pipet 5.0 mL of this solution, and add water to make exactly 250 mL. Pipet 1.0 mL of this solution, add 5.0 mL of 1 mol/L sodium hydroxide TS and 1.0 mL of the internal standard solution, centrifuge if necessary, and use the supernatant liquid as the standard solution. Perform the test with 1 μL each of the test solution and standard

solution as directed under Gas Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of dimethylaniline to that of the internal standard (not more than 20 ppm).

$$\text{Content (ppm) of dimethylaniline} = \frac{Q_T}{Q_S} \times \frac{\text{Amount (mg) of dimethylan iline taken} \times \text{Content (\%) of dimethylan iline}}{\text{Amount (mg) of Piperacill in Sodium taken}} \times 4$$

Internal standard solution—Dissolve 50.0 mg of naphthalene in cyclohexane to make 50 mL. To 5.0 mL of this solution add cyclohexane to make 100 mL, and use this solution as the internal standard solution.

Operating conditions

Detector: A hydrogen flame ionization detector

Column: A column, about 2 mm in internal diameter and 2 m in length, packed with diatomaceous earth for gas chromatography, coated with 50 % phenyl-50 % methylpolysiloxane for gas chromatography at the rate of 3 %.

Column temperature: 120 °C

Injection port and detector temperature: 150 °C

Carrier gas: Nitrogen

Flow rate: 30 mL/minute

Water Not more than 1.0 % (3 g, volumetric titration, direct titration).

Sterility Test It meets the requirement, when Piperacillin Sodium is used in a sterile preparation.

Bacterial Endotoxins Less than 0.07 EU/mg (potency) of piperacillin, when Piperacillin Sodium is used in a sterile preparation.

Assay Weigh accurately about 0.1 g (potency) of Piperacillin Sodium, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the test solution. Separately, weigh accurately an amount of Piperacillin RS, equivalent to about 0.1 g (potency), and dissolve in the mobile phase to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak height of piperacillin to that of the internal standard.

$$\text{Amount [μg (potency)] of piperacillin } (C_{23}H_{26}N_5O_7S) = \text{Amount [μg (potency)] of Piperacillin RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of acetanilide in the mobile phase (1 in 5000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column, about 4.6 mm in internal diameter and about 150 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter)

Column temperature: A constant temperature of about 25 °C

Mobile phase: To 60.1 g of acetic acid (100) and 101.0 g of triethylamine add water to make exactly 1000 mL. To 25 mL of this solution add 25 mL of dilute acetic acid and 210 mL of acetonitrile, and add water to make exactly 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of piperacillin is about 5 minutes.

System suitability

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the internal standard and piperacillin are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak height of piperacillin to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Piperacillin Sodium for Injection

Piperacillin Sodium for Injection is a preparation for injection which is dissolved before use, and contains not less than 93.0 % and not more than 107.0 % of the labeled amount of piperacillin ($C_{23}H_{27}N_5O_7S$: 517.55).

Method of Preparation Prepare as directed under Injections, with Piperacillin Sodium.

Description Piperacillin Sodium for Injection appears as white powder or masses.

Identification Proceed as directed under the Identification (1) and (2) under Piperacillin Sodium.

pH Dissolve an amount of Piperacillin Sodium for Injection, equivalent to 1.0 g (potency) of piperacillin sodium according to the labeled amount, in 4 mL of water: the pH of this solution is between 5.0 and 7.0.

Purity (1) ***Clarity and color of solution***—Dissolve an amount of Piperacillin Sodium for Injection, equivalent to 4.0 g (potency) of piperacillin sodium according to the labeled amount, in 17 mL of water: the solution is clear and colorless.

(2) ***Related substances***—Proceed as directed in Identification (4) under Piperacillin Sodium.

Water Not more than 1.0 % (3 g, volumetric titration, direct titration).

Sterility Test It meets the requirement, when Piperacillin Sodium for Injection is used in a sterile preparation.

Bacterial Endotoxins Less than 0.04 EU/mg (potency) of piperacillin.

Uniformity of Dosage Units It meets the requirement of the Mass Variation Test.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

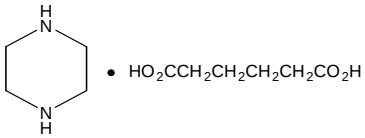
Assay Weigh accurately an amount of the contents of not less than 10 containers of Piperacillin Sodium for Injection, equivalent to about 20 mg (potency) of Piperacillin Sodium according to the labeled amount, and dissolve in water to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the test solution. Separately, weigh accurately an amount of Piperacillin RS, equivalent to about 20 mg (potency), and dissolve in the mobile phase to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Proceed as directed in the operating conditions in the Assay under Piperacillin Sodium.

$$\text{Amount [μg (potency)] of piperacillin } (C_{23}H_{26}N_5O_7S) = \text{Amount [μg (potency)] of Piperacillin RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of acetanilide in the mobile phase (1 in 5000)

Containers and Storage ***Containers***—Hermetic containers. Plastic containers for aqueous injections may be used.

Piperazine Adipate



$C_4H_{10}N_2 \cdot C_6H_{10}O_4$: 232.28

Piperazine hexanedioate [*142-88-1*]

Piperazine Adipate, when dried, contains not less than 98.5 % and not more than 101.0 % of piperazine adipate ($C_4H_{10}N_2 \cdot C_6H_{10}O_4$).

Description Piperazine Adipate is a white, crystalline powder, is odorless and has a slightly acid taste.
Piperazine Adipate is soluble in water or in acetic acid (100) and practically insoluble in ethanol (95), in acetone or in ether.

Melting point—About 250 °C (with decomposition).

Identification (1) Dissolve 0.5 g of Piperazine Adipate in 10 mL of water, add 1 mL of hydrochloric acid and extract with two 20 mL volumes of ether. Combine the ether extracts, evaporate to dryness in a water-bath and dry the residue at 105 °C for 1 hour: the melting point of the residue is between 152 °C and 155 °C.

(2) Take 3 mL of a solution of Piperazine Adipate (1 in 100) and add 3 drops of Reinecke salt TS: a pale red precipitate is formed.

(3) Determine the infrared spectra of Piperazine Adipate and Piperazine Adipate RS, previously dried, as directed in the potassium bromide disk method underlnfrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

pH The pH of a solution obtained by dissolving 1 g of Piperazine Adipate in 20 mL of water is between 5.0 and 6.0.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Piperazine Adipate in 30 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 2.0 g of Piperazine Adipate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Weigh accurately 1 g of Piperazine Adipate, dissolve in the diluent to make exactly 10 mL, and use this solution as the test solution (1). Pipet 1.0 mL of the test solution (1), dissolve in the diluent to make exactly 10 mL, and use this solution as the test solution (2). Separately, weigh accurately 0.1 g of Piperazine Adipate RS, dissolve in the diluent to make exactly 10 mL, and use this solution as the standard solution (1). Weigh accurately 25 mg of ethylenediamine, dissolve in the diluent to make exactly 100 mL, and use this solution as the standard solution (2). Weigh accurately 25 mg of triethylenediamine, dissolve in the diluent to make exactly 100 mL, and use this solution as the standard solution (3). Weigh accurately 25 mg of triethylenediamine and 1.0 g of Piperazine Adipate RS, dissolve in the diluent to make exactly 100 mL, and use this solution as the standard solution (4). Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL

each of the test solutions (1) and (2) and standard solutions (1), (2), (3), and (4) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone and 13.5 mol/L ammonia water (80 : 20) to a distance of about 15 cm, and dry the plate at 105 °C. Spray evenly a 0.3 % solution of ninhydrin in a mixture of 1-butanol and acetic acid (100) (100 : 3) on the plate, spray a 0.15 % solution of ninhydrin in anhydrous alcohol on the plate, and dry at 105 °C for 10 minutes: any spot other than the principal spot obtained from the test solution (1) is not more intense than the principal spot from standard solution (2) (not more than 0.25 %). Spray iodine TS on the plate, and allow to stand for 10 minutes: the spot corresponding to triethylenediamine from the test solution (1) is not more intense than the spot from the standard solution (3) (not more than 0.25 %). This test is not valid unless the principal spot and the spot corresponding to triethylenediamine from the standard solution (4) are clearly resolved.

Diluent—A mixture of 13.5 mol/L ammonia water and anhydrous ethanol (3 : 2)

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 4 hours).

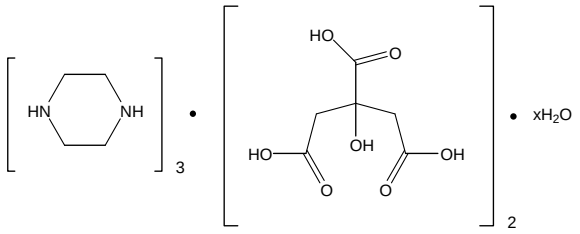
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.2 g of Piperazine Adipate, previously dried, dissolve in a mixture of 20 mL of acetic acid (100) and 40 mL of acetone for nonaqueous titration and titrate with 0.1 mol/L perchloric acid VS until the red-purple color of the solution changes to blue-purple (indicator: 6 drops of bromocresol green-methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 11.614 mg of $C_4H_{10}N_2 \cdot C_6H_{10}O_4$

Containers and Storage ***Containers***—Well-closed containers.

Piperazine Citrate Hydrate



$(C_4H_{10}N_2)_3 \cdot 2C_6H_8O_7 \cdot xH_2O$

Piperazine 2-2-hydroxypropane-1,2,3-tricarboxylate hexahydrate [41372-10-5]

Piperazine Citrate Hydrate contains not less than 98.0 % and not more than 100.5 % of piperazine citrate $[(C_4H_{10}N_2)_3 \cdot 2C_6H_8O_7$: 642.65], calculated on the anhydrous basis.

Description Piperazine Citrate Hydrate appears as white crystalline powder and has a slight odor.
Piperazine Citrate Hydrate is soluble in water and very slightly soluble in ethanol (95) or in ether.

pH—The pH of a solution of Piperazine Citrate Hydrate (1 in 10) is about 5.

Identification (1) Dissolve 0.2 g of Piperazine Citrate Hydrate in 5 mL of 3 mol/L hydrochloric acid and add, with stirring, 1 mL of sodium nitrite VS (1 in 2). Cool in a water-bath for 15 minutes, stirring, if necessary, to induce crystallization, filter the precipitate on a sintered-glass funnel, wash with 10 mL of cold water and dry at 105 °C: the obtained *N,N'*-dinitrosopiperazine melts between 156 °C and 160 °C.

(2) Piperazine Citrate Hydrate responds to the Qualitative Tests for citrate.

Purity (1) ***Primary amines and ammonia***—Dissolve 0.5 g of Piperazine Citrate Hydrate in 10 mL of water. Add 1 mL of 2.5 mol/L sodium hydroxide, 1 mL of acetone and 1 mL of sodium nitropruside TS. Mix and allow to stand for exactly 10 minutes. Determine, A_1 and A_2, the absorbance of this solution at 520 nm and at 600 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry, using a blank consisting of the same quantities of the same reagents, but substituting water for the sodium hydroxide solution. The ratio, A_2/A_1, is not more than 0.5 (not more than about 0.7 % of primary amines and ammonia).

(2) ***Heavy metals***—Dissolve 1.25 g of Piperazine Citrate Hydrate in water to make 25 mL, and use this solution as the test solution. Separately, to 3 mL of standard lead solution add water to make 30 mL. To 10 mL of this solution add 2 mL of the test solution, and use this solution as the control solution. To 10 mL of water add 2 mL of the test solution, and use this solution as the blank solution. To 12 mL each of the test solution, control solution, and blank solution add 2 mL of pH 3.5 acetate buffer solution, mix, add 1.2 mL of thioacetamide TS, and mix immediately. Allow to stand for 2 minutes: the color of the test solution is not more intense than that of the control solution (not more than 20 ppm).

System suitability: The control solution shows a slightly brown color compared to the blank solution.

(3) ***Related substances***—Weigh accurately 1 g of Piperazine Citrate Hydrate, dissolve in the diluent to make exactly 10 mL, and use this solution as the test solution (1). Pipet 1.0 mL of the test solution (1), dis-

solve in the diluent to make exactly 10 mL, and use this solution as the test solution (2). Separately, weigh accurately 0.1 g of Piperazine Citrate RS, dissolve in the diluent to make exactly 10 mL, and use this solution as the standard solution (1). Weigh accurately 25 mg of ethylenediamine, dissolve in the diluent to make exactly 100 mL, and use this solution as the standard solution (2). Weigh accurately 25 mg of triethylenediamine, dissolve in the diluent to make exactly 100 mL, and use this solution as the standard solution (3). Weigh accurately 25 mg of triethylenediamine and 1.0 g of Piperazine Citrate RS, dissolve in the diluent to make exactly 100 mL, and use this solution as the standard solution (4). Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solutions (1) and (2) and standard solutions (1), (2), (3), and (4) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone and 13.5 mol/L ammonia water (80 : 20) to a distance of about 15 cm, and dry the plate at 105 °C. Spray evenly a 0.3 % solution of ninhydrin in a mixture of 1-butanol and acetic acid (100) (100 : 3) on the plate, spray a 0.15 % solution of ninhydrin in anhydrous alcohol on the plate, and dry at 105 °C for 10 minutes: any spot other than the principal spot obtained from the test solution (1) is not more intense than the principal spot from the standard solution (2) (not more than 0.25 %). Spray iodine TS on the plate, and allow to stand for 10 minutes: the spot corresponding to triethylenediamine from the test solution (1) is not more intense than the spot from the standard solution (3) (not more than 0.25 %). This test is not valid unless the principal spot and the spot corresponding to triethylenediamine from the standard solution (4) are clearly resolved.

Diluent—A mixture of 13.5 mol/L ammonia water and anhydrous ethanol (3 : 2)

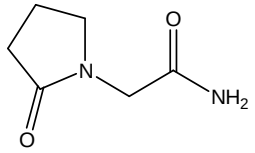
Water Not more than 12.0 % (0.3 g, direct titration).

Assay Weigh accurately about 0.2 g of Piperazine Citrate Hydrate, and dissolve in 100 mL of acetic acid (100). Warm, if necessary, and titrate with 0.1 mol/L perchloric acid VS (indicator: 3 drops of methylrosaniline cholride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 10.711 mg of $(C_4H_{10}N_2)_3 \cdot 2C_6H_8O_7$

Containers and Storage ***Containers***—Well-closed containers.

Piracetam



$C_6H_{10}N_2O_2$: 142.16

2-(2-Oxopyrrolidin-1-yl)acetamide [*72496-41-4*]

Piracetam contains not less than 98.0 % and not more than 102.0 % of piracetam, calculated on the anhydrous basis.

Description Piracetam is white powder.
Piracetam is freely soluble in water, and soluble in ethanol (95).
Piracetam shows polymorphism.

Identification Determine the infrared spectra of Piracetam and Piracetam RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any differences appear, dissolve Piracetam and Piracetam RS in ethanol (95), evaporate in water-bath to dryness, respectively and with the residues repeat the test.

Purity (1) ***Clarity and color of solution***—A solution of 2.0 g of Piracetam in 10 mL of water is clear and colorless.

(2) ***Heavy metals***—Proceed with 2.0 g of Piracetam according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Dissolve 50.0 mg of Piracetam in a mixture of water and acetonitrile (90 : 10) to make exactly 100 mL and use this solution as the test solution. Separately, dissolve 5 mg of Piracetam and 10 mg of piracetam related substance I RS {2-pyrrolidone} in a mixture of water and acetonitrile (90 : 10) to make exactly 100 mL and use this solution as the standard solution (1). To 1.0 mL of the test solution, add a mixture of water and acetonitrile (90 : 10) to make exactly 100 mL, to 5.0 mL of this solution, add a mixture of water and acetonitrile (90 : 10) to make exactly 50 mL and use this solution as the standard solution (2). Perform the test with 20 μL each of the test solution and standard solution (2) as directed under Liquid Chromatography, according to the following operating conditions: the areas of any peaks except the principal peak obtained from the test solution are not more than the area of the principal peak from the standard solution (2) (0.1 %), and the total area of peaks except the principal peak from the test solution is not more than 3 times the area of the principal peak from the standard solution (2) (0.3 %). The peaks with area of less than 0.5 times the area of the principal peak

from the standard solution (2).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 205 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter)

Mobile phase: A mixture of the phosphate and acetonitrile (90 : 10)

Flow rate: 1.0 mL/minute.

System suitability

System performance: When the procedure is run with the standard solution (1) under the above operating conditions, the resolution between the peaks of piracetam and piracetam related substance I is not less than 3.0 and the symmetry factor of the piracetam peak is not more than 2.0.

Time span of measurement: About 8 times as long as the retention time of piracetam.

Phosphate buffer—Dissolve 1.0 g of potassium dihydrogen phosphate in water to make 1000 mL and adjust to pH 6.0 with dilute phosphoric acid.

Loss on Drying Not more than 1.0 % (1.0 g, 105 ℃, constant mass).

Residue on Ignition Not more than 0.1 % (1 g).

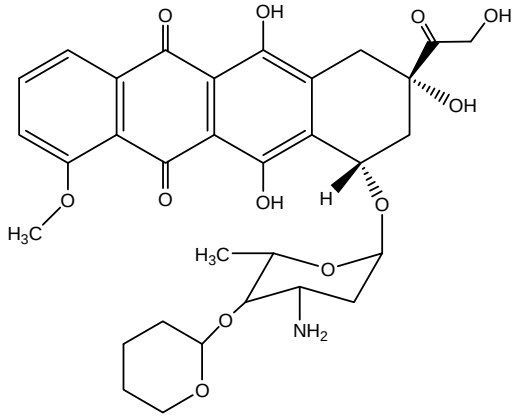
Assay Weigh accurately about 0.75 g of Piracetam, dissolve in 50 mL of water, add 20.0 mL of 1 mol/L sodium hydroxide VS, and heat to boil for 15 minutes. Cool, add 25.0 mL of 1 mol/L hydrochloric acid VS, and heat to boil for 2 minutes. Cool and and titrate with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 1 mol/L sodium hydroxide VS
= 142.15 mg of $C_6H_{10}N_2O_2$.

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Pirarubicin



$C_{32}H_{37}NO_{12}$: 627.64

(8*S*,10*S*)-10-{[(2*R*,4*S*,5*S*,6*S*)-4-Amino-6-methyl-5-{[(*S*)-tetrahydro-2*H*-pyran-2-yl]oxy}tetrahydro-2*H*-pyran-2-yl]oxy}-6,8,11-trihydroxy-8-(2-hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione [*72496-41-4*]

Pirarubicin is a derivative of daunorubicin.
Pirarubicin contains not less than 950 μg (potency) per mg of pirarubicin, calculated on the anhydrous basis.

Description Pirarubicin appears as red-brown crystalline powder.
Pirarubicin is soluble in chloroform, very slightly soluble in acetonitrile, in methanol, or in ethanol (99.5), and practically insoluble in water.

Identification (1) Dissolve 10 mg of Pirarubicin in 80 mL of methanol and 6 mL of diluted hydrochloric acid (1 in 5000), and add water to make 100 mL. To 10 mL of this solution add diluted methanol (4 in 5) to make 100 mL. Determine the absorbances of this solution and a solution of Pirarubicin RS prepared in the same manner as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve separately 5 mg each of Pirarubicin and Pirarubicin RS in 5 mL of chloroform, and use these solutions as the test solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (5 : 1) to a distance of about 10 cm, and air-dry the plate: the principal spot obtained from the test solution and the spot from the standard solution show a red-brown color and the same R_f value.

Specific Optical Rotation $[\alpha]_D^{20}$: +195 ~ +215° (10 mg, chloroform, 10 mL, 100 mm).

Absorbance $E_{1cm}^{1\%}$ (495 nm): 195 ~ 220. Weigh accurately about 10 mg (potency) of Pirarubicin, dissolve in 80 mL of methanol and 6 mL of diluted hydrochloric acid (1 in 5000), and add water to make exactly 100 mL. Pipet 10 mL of this solution, add a solution of methanol (4 in 50) to make exactly 50 mL, and determine the absorbance at 495 nm as directed under Ultraviolet-visible Spectrophotometry.

Purity (1) ***Clarity and color of solution***—Dissolve 10 mg of Pirarubicin in 10 mL of 0.01 mol/L hydrochloric acid TS: the solution is clear and red in color.

(2) ***Heavy metals***—Proceed with 1.0 g of Pirarubicin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Dissolve 10 mg of Pirarubicin in 20 mL of the mobile phase, and use this solution as the test solution. Pipet 1 mL of this solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method: the peak area of dororubicin, having the relative retention time of about 0.45 with respect to pirarubicin, and the area of the peak, having the relative retention time of about 1.2 with respect to pirarubicin, are not larger than the peak area of pirarubicin from the standard solution, respectively, and the sum of the areas of the peaks, having the relative retention times of about 1.9 and about 2.0 with respect to pirarubicin, is not larger than 5 times the peak area of pirarubicin from the standard solution. Use the peak area of doxorubicin after multiplying by its relative response factor, 0.94, and the areas of the peaks, having the relative retention times of about 1.9 and about 2.0, after multiplying by their relative response factors, 1.09, respectively.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability

System performance and system repeatability: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Pipet 2 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of pirarubicin obtained from 20 μL of this solution is equivalent to 14 to 26 % of that from the standard solution.

Time span of measurement: About 4 times as long as the retention time of pirarubicin.

Water Not more than 2.0 % (0.1 g, volumetric titration, direct titration).

Sterility Test It meets the requirement, when Pirarubicin is used in a sterile preparation.

Bacterial Endotoxins Less than 2.50 EU/mg (potency) of pirarubicin, when Pirarubicin is used in a sterile preparation.

Histamine It meets the requirement, when Pirarubicin is used in a sterile preparation. Dissolve a suitable amount of Pirarubicin in diluted hydrochloric acid (1 in 3800) so that each mL contains 2.0 mg (potency), dilute a suitable amount of this solution 100-fold with water, and use this solution as the test solution. Use 0.5 mL of the test solution.

Assay Weigh accurately about 10 mg (potency) each of Pirarubicin and Pirarubicin RS, and dissolve each in the mobile phase to make exactly 10 mL. To 5 mL each of these solutions add exactly 5 mL of the internal standard, and use these solutions as the test solution and standard solution, respectively. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography, and calculate the ratios, Q_T and Q_S, of the peak area of pirarubicin to that of the internal standard in each solution.

$$\text{Amount [μg (potency)] of pirarubicin } (C_{32}H_{37}NO_{12}) = \text{Amount [μg (potency)] of Pirarubicin RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of 2-naphthol in the mobile phase (1 in 1000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: A mixture of 0.05 mol/L ammonium formate buffer solution (pH 4.0) and acetontrile (3 : 2)

Flow rate: Adjust the flow rate so that the retention time of pirarubicin is about 7 minutes.

System suitability

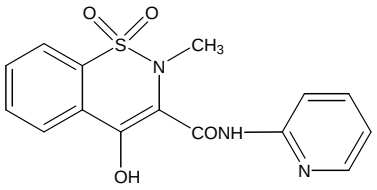
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, pirarubicin and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard

deviation of the ratio of the peak area of pirarubicin to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Piroxicam



$C_{15}H_{13}N_3O_4S$: 331.35

(3*E*)-3[Hydroxy-(pyridin-2-ylamino)methylidene]-2-methyl-1,1-dioxo-3,4-dihydro-1*H*-[1,2]benzothiazin-4-one [*36322-90-4*]

Piroxicam contains not less than 97.0 % and not more than 103.0 % of piroxicam ($C_{15}H_{13}N_3O_4S$), calculated on the anhydrous basis.

Description Piroxicam is a grayish white to pale brown or pale yellow powder and is odorless.
Piroxicam is sparingly soluble in acetic anhydride, slightly soluble in acetonitrile, in methanol or in ethanol (99.5), very slightly soluble in acetic acid (100), and practically insoluble in water.

Identification (1) Weigh 0.1 g of Piroxicam, dissolve in a mixture of chloroform and methanol (1 : 1) to make 100 mL and use this solution as the test solution. Separately, weigh 0.1 g of Piroxicam RS, follow the procedure for the preparation of the test solution and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 20 μL each of the test solution and the standard solution on a plate (thickness: 0.25 mm) of silica gel for thin-layer chromatography. Develop the plate with a mixture of toluene and acetic acid (100) (95 : 5) to a distance of about 15 cm, air-dry the plate, develop again the plate in the above solution as before, mark the solvent front and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot from the test solution shows the same R_f value as the principal spot from the standard solution.

(2) Determine the absorption spectra of solutions of Piroxicam and Piroxicam RS, respectively, in methanolic hydrochloric acid (1 in 1200) solution (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit maxima and minima at the same wavenumbers.

(3) Determine the infrared spectra of Piroxicam and Piroxicam RS, as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity ***Heavy metals***—Proceed with about 1.0 g of Piroxicam according to the Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

Water Not more than 0.5 %. (1 g, volumetric titration, direct titration)

Residue on Ignition Not more than 0.3 % (1 g).

Assay Dissolve about 50 mg of Piroxicam, accurately weighed, in 0.01 mol/L methanolic hydrochloric acid to make 100 mL exactly. Transfer 10.0 mL of the solution to the 100 mL volumetric flask, add 50 mL of 0.01 mol/L methanolic hydrochloric acid and 20.0 mL of water, add 0.01 mol/L methanolic hydrochloric acid to make exactly 100 mL, mix, and use this solution as the test solution. Separately, weigh accurately about 50 mg of Piroxicam RS, dissolve in 0.01 mol/L methanolic hydrochloric acid to make exactly 100 mL. Transfer 10.0 mL of this solution to the 100 mL volumetric flask, add 50 mL of 0.01 mol/L methanolic hydrochloric acid and 20.0 mL of water, add 0.01 mol/L methanolic hydrochloric acid to make exactly 100 mL, mix, and use this solution as the standard solution. Perform the test with 25 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the main peak areas, A_T and A_S, of the test solution and the standard solution, respectively.

$$\text{Amount (mg) of piroxicam } (C_{15}H_{13}N_3O_4S) = \text{Amount (mg) of Piroxicam RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 3.9 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 to 10 μm in particle diameter).

Mobile phase: A mixture of the buffer solution and methanol (55 : 45).

System suitability

System performance: When the procedure is run with 25 μL of the standard solution according to the above operating conditions, the symmetry factor of the peak is not more than 1.5.

System repeatability: When the test is repeated 6 times with 25 μL each of the standard solution according to the above operating conditions, the relative standard deviation of the peak areas of Piroxicam is not more than 2.0 %.

Buffer solution—Dissolve 7.72 g of anhydrous citric acid in 400 mL of water and separately dissolve 5.35 g of dibasic sodium phosphate in 100 mL of water.

Add the phosphate solution to the citric acid solution, dilute with water to make 1000 mL and mix.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Piroxicam Capsules

Piroxicam Capsules contain not less than 92.5 % and not more than 107.5 % of the labeled amount of piroxicam ($C_{15}H_{13}N_3O_4S$: 331.35).

Method of Preparation Prepare as directed under Capsules, with Piroxicam.

Identification Powder the contents of the Piroxicam Capsules, weigh a portion of the powder, equivalent to 20 mg of Piroxicam according to the labeled amount, add 20 mL of a mixture of chloroform and methanol (1 : 1), shake for 10 minutes, filter and use the filtrate as the test solution. Proceed as directed in the Identification (1) under Piroxicam.

Water Not more than 8.0 %. (0.3 g, volumetric titration, direct titration)

Dissolution Test Perform the test with 1 tablet of Piroxicam Capsules at 50 revolutions per minute according to Method 1 under the Dissolution Test, using 900 mL of the 1st fluid for disintegration test as a dissolution solution. Take the dissolved solution after 45 minutes from the start of the test, filter, dilute properly with a dissolution solution, if necessary, and use this solution as the test solution. Separately, weigh accurately a portion of Piroxicam RS, add methanol to obtain a solution having a known concentration of about 0.5 mg per mL and use this solution as the stock solution. Take a proper portion of the stock solution exactly, dilute exactly with a dissolution solution to make a known concentration and use this solution as the standard solution. Determine the absorbances of the test solution and the standard solution at the wavelength of a maximum absorbance at about 333 nm.
The dissolution rate of Piroxicam Capsules in 45 minutes is not less than 75 %.

Uniformity of Dosage Units It meets the requirement.

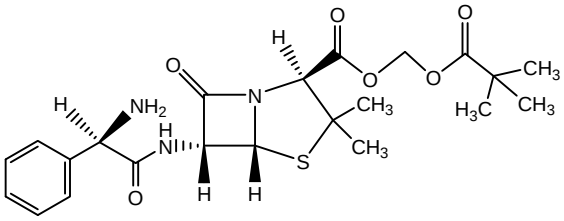
Assay Weigh accurately the mass of the contents of not less than 20 Proxicam Capsules, transfer as completely as possible to a suitable container and determine the average weight per capsule. Mix the combined contents and transfer an accurately weighed portion, equivalent to about 50 mg of piroxicam ($C_{15}H_{13}N_3O_4S$) according to labeled amount, to 100 mL of a volumetric flask. Add 70 mL of 0.01 mol/L methanolic hydrochloric acid and mix with sonicator for 30 minutes. Dilute with 0.01 mol/L methanolic hydrochloric acid to make 100 mL and mix. Centrifuge this solution to obtain a clear solution. Transfer 10.0 mL of the solution so obtained to a volumetric flask, add 50 mL of 0.01 mol/L methanolic hydrochloric acid and 20.0 mL of water and add 0.01 mol/L methanolic hydrochloric acid to make 100 mL, mix and use this solution as the test solution. Proceed as directed in the Assay under Piroxicam.

$$\text{Amount (mg) of piroxicam } (C_{15}H_{13}N_3O_4S) = \text{Amount (mg) of Piroxicam RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Pivampicillin



$C_{22}H_{29}N_3O_6S$: 463.55

2,2-Dimethylpropanoyloxymethyl(2*S*,5*R*,6*R*)-6-{[(2*R*)-2-amino-2-phenylacetyl]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate
[*33817-20-8*]

Pivampicillin contains not less than 970 μg (potency) per mg of pivampicillin ($C_{22}H_{29}N_3O_6S$: 463.55), calculated on the anhydrous basis.

Description Pivampicillin appears as white crystalline powder, and is odorless and tasteless.
Pivampicillin is freely soluble in acetonitrile, soluble in ethanol (95) or in methanol, slightly soluble in ether, and practically insoluble in water.

Identification Determine the infrared spectra of Pivampicillin and Pivampicillin RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +210 ~ +220° (0.1 g, a solution prepared by dissolving in 5 mL of anhydrous ethanol and adding 0.01 mol/L hydrochloric acid TS to make 10 mL, 100 mm).

Meltin Point 115 ~ 117 °C.

pH Dissolve 1.0 g (potency) of Pivampicillin in 10 mL of water: the pH of this solution is between 6.0 and 8.0.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Pivampicillin according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

(2) ***Related substances***—Weigh accurately 50 mg of Pivampicillin, dissolve in 10 mL of acetonitrile, add a solution of phosphoric acid (1 in 1000) to make exactly 20 mL, and use this solution as the test solution. Pipet 2.0 mL of the test solution, add 9.0 mL of acetonitrile and 9.0 mL of a solution of phosphoric acid (1 in 1000), and use this solution as the standard solution. Perform the test with 50 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method: the sum of the areas of the paks of related substances obtained from the test solution is not larger than 0.3 times the area of the principal peak from the standard solution (not more than 3 %). Exclude any peak with an area less than 0.01 time the area of the principal peak from the standard solution (0.1 %).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 12.5 cm in length, packed with octylsilyl silica gel for chromatography (5 μm in particle diameter).

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: A mixture of a solution of ammonium phosphate (1.32 in 1000) adjusted to pH 2.5 with 100 g/L phosphoric acid solution, and acetonitrile (50 : 50)

Mobile phase B: A mixture of a solution of ammonium phosphate (1.32 in 1000) adjusted to pH 2.5 with 100 g/L phosphoric acid solution, and acetonitrile (15 : 85)

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-10	100	0
10-12	0	100
12-17	100	0

Flow rate: 1.5 mL/minute

System suitability

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the retention time of the pivampicillin dimer is about 5 minutes, and the capacity factor is not less than 12.

(3) ***Dimethylaniline***—Weigh accurately about 1.0 g of Pivampicillin, add 5 mL of 1 mol/L sodium hydroxide TS and 1 mL of the internal standard solution, centrifuge if necessary, and use the clear supernatant liquid as the test solution. Separately, weigh accurately about 50 mg of dimethylaniline, add 2.0 mL of hydrochloric acid, and add water to make 50 mL. Pipet 5.0 mL of this solution, and add water to make exactly 250 mL. Pipet 1.0 mL of this solution, add 5.0 mL of 1 mol/L sodium hydroxide TS and 1.0 mL of the internal standard solution, centrifuge if necessary, and use the clear supernatant liquid as the standard solution. Perform the test with 1 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of dimethylaniline to that of the internal standard in each solution (not more than 20 ppm).

(4) ***2,2',2"-Nitrilotriethanol***—Weigh accurately 0.1 g of Pivampicillin, dissolve in 1 mL of a mixture of water and acetonitrile (1 : 9), and use this solution as the test solution. Weigh accurately 5 mg of 2,2',2"-nitrilo-triethanol, add a mixture of water and acetonitrile (1 : 9) to make 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of butyl acetate, acetic acid (100), phosphate buffer solution (pH 5.81), butanol, and methanol (80 : 40 : 24 : 15 : 5) to a distance of about 12 cm, dry the plate at 110 °C for 10 minutes, and cool. Place a mixture of a solution of potassium permanganate (15 in 1000), hydrochloric acid, and water (2 : 1 : 1) in an evaporating dish, put the dish at the bottom of the chamber for thin-layer chromatography and close the chamber for about 15 minutes. Place the dried plate in the chamber for thin-layer chromatography, close the chamber, and allow to stand in chlorine vapor for 15 to 20 minutes. Remove the plate, allow to stand for 2 to 3 minutes to remove the vapor, and spray tetramethyldiaminodiphenylmethane TS. The spot corresponding to 2,2',2"-nitrilotriethanol obtained from the test solution is not more intense than the spot from the standard solution (not more than 0.05 %).

Water Not more than 0.5 % (1 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 50 mg (potency) each of Pivampicillin and Pivampicillin RS, and dissolve each in water to make exactly 50 mL. Pipet 10.0 mL each of these solutions, add the mobile phase to make exactly 50.0 mL, and use these solutions as the test

solution and standard solution, respectively. Use the test solution and standard solution within 2 hours after preparation. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of pivampicillin in each solution.

$$\text{Amount [μg (potency)] of pivampicillin } (C_{22}H_{29}N_3O_6S) = \text{Amount [μg (potency)] of Pivampicillin RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 12.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of 2.22 g/L phosphoric acid solution (adjusted to pH 2.5 with triethylamine) and acetonitrile (3 : 2)

Flow rate: 1.5 mL/minute

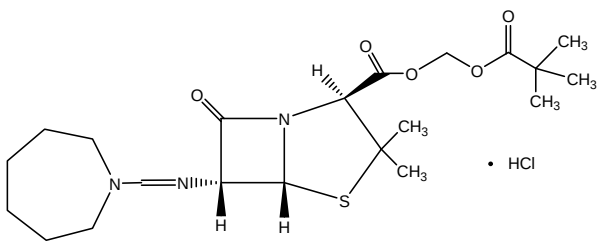
System suitability

System performance: Dissolve 25.0 mg of Propyl Paraoxybenzoate RS in the mobile phase to make 50.0 mL, and to 10.0 mL of this solution add the mobile phase to make exactly 50.0 mL. Mix 5.0 mL of this solution with 5.0 mL of the standard solution. When the procedure is run with 20 μL of this solution under the above operating conditions, pivampicillin and propyl paraoxybenzoate are eluted in this order with the resolution between these peaks being not less than 5.0.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pivampicillin is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Pivmecillinam Hydrochloride



$C_{21}H_{33}N_3O_5S \cdot HCl$: 476.03

2,2-Dimethylpropanoyloxymethyl (2*S*,5*R*,6*R*)-6-[(azepan-1-ylmethylidene)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate hydrochloride [*32887-03-9*]

Pivmecillinam Hydrochloride contains not less than 630 μg (potency) and not more than 710 μg (potency) per mg of mecillinam ($C_{15}H_{23}N_3O_3S$: 325.43), calculated on the anhydrous basis.

Description Pivmecillinam Hydrochloride appears as white to yellowish white crystalline powder.
Pivmecillinam Hydrochloride is very soluble in methanol or in acetic acid (100), freely soluble in water or in ethanol (99.5), and soluble in acetonitrile.

Identification (1) Determine the infrared spectra of Pivmecillinam Hydrochloride and Pivmecillinam Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dissolve 0.5 mg of Pivmecillinam Hydrochloride in 10 mL of water, and add 1 mL of dilute nitric acid and 1 drop of silver nitrate TS: a white precipitate is produced.

Specific Optical Rotation $[\alpha]_D^{20}$: +200 ~ +220° (1.0 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.5 g of Pivmecillinam Hydrochloride in 10 mL of water is between 3.0 and 4.5.

Purity (1) ***Heavy metals***—To 1.0 g of Pivmecillinam Hydrochloride add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), fire the ethanol to burn, and heat gradually to incinerate. If a carbonized substance remains, moisten with a small amount of nitric acid, and ignite to incinerate. After cooling, add 3 mL of hydrochloric acid to the residue, warm in a water bath to dissolve, and evaporate to dryness. To the residue add 10 mL of water, warm in a water bath to dissolve, cool, adjust the pH to 3 to 4 by adding ammonia TS dropwise, add 2 mL of dilute acetic acid, filter if necessary, wash with 10 mL of water, transfer the filtrate and washing to a Nessler tube, and add water to make 50 mL. Use this solution as the test solution. Prepare the control solution with 2.0 mL of standard lead solution in the same manner as the test solution (not more than 20 ppm).

(2) ***Arsenic***—Prepare the test solution with 1.0 g of Pivmecillinam Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

(3) ***Related substances***—Weigh accurately 50 mg of Pivmicillinam Hydrochloride, dissolve in 4.0 mL of a mixture of acetonitrile and acetic acid (100) (97 : 3), and use this solution as the test solution. Separately, weigh accurately 2.0 mg of Pivmecillinam Hydrochloride RS, dissolve in 4.0 mL of water, and use this solution as the standard solution. Perform the test with the-

se solutions as directed under Thin-layer Chromatography. Spot 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography, allow to stand for 30 minutes, and spot 2 μL of the test solution. Develop the plate with a mixture of acetone, water, and acetic acid (100) (10 : 1 : 1) to a distance of about 12 cm, and air-dry the plate. Allow to stand in iodine vapor for 10 minutes: the spot obtained from the test solution is not larger or more intense than the spot from the standard solution. Any spot from the test solution is not observed in any position other than the position corresponding to the spot from the standard solution.

Dimethylaniline—Weigh accurately about 1.0 g of Pivmecillinam Hydrochloride, add 5 mL of 1 mol/L sodium hydroxide TS and 1 mL of the internal standard solution, centrifuge if necessary, and use the clear supernatant liquid as the test solution. Separately, weigh accurately about 50 mg of dimethylaniline, and add 2.0 mL of hydrochloric acid and water to make 50 mL. Pipet 5.0 mL of this solution, and add water to make exactly 250 mL. Pipet 1.0 mL of this solution, add 5.0 mL of 1 mol/L sodium hydroxide TS and 1.0 mL of the internal standard solution, centrifuge if necessary, and use the clear supernatant liquid as the standard solution. Perform the test with 1 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of dimethylaniline to that of the internal standard in each solution (not more than 20 ppm).

$$\text{Content (ppm) of dimethylaniline} = \frac{Q_T}{Q_S} \times \frac{\text{Amount (mg) of dimethylaniline taken} \times \text{Content (\%) of dimethylaniline}}{\text{Amount (mg) of Pivmecillinam Hydrochloride taken}} \times 4$$

Internal standard solution—Dissolve 50.0 mg of naphthalene in cyclohexane to make 50 mL. To 5.0 mL of this solution add cyclohexane to make 100 mL, and use this solution as the internal standard solution.

Operating conditions

Detector: A hydrogen flame ionization detector

Column: A column, about 2 mm in internal diameter and 2 m in length, packed with diatomaceous earth for gas chromatography, coated with 50 % phenyl-50 % methylpolysiloxane for gas chromatography at the rate of 3 %.

Column temperature: 120 °C

Injection port and detector temperature: 150 °C

Carrier gas: Nitrogen

Flow rate: 30 mL/minute

Water Not more than 1.0 % (0.25 g, coulometric titration).

Assay Weigh accurately an amount each of Pivmecillinam Hydrochloride and Pivmecillinam Hydrochloride RS, equivalent to about 20 mg (potency), dissolve each in a suitable volume of the mobile phase, add exactly 10 mL each of the internal standard solution, add the mobile phase to make exactly 100 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of pivmecillinam to that of the internal standard in each solution.

$$\text{Amount [μg (potency)] of mecillinam } (C_{15}H_{23}N_3O_3S) = \text{Amount [μg (potency)] of Pivmecillinam Hydrochloride RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of diphenyl in the mobile phase (1 in 12500)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 0.771 g of ammonium acetate in about 900 mL of water, adjust the pH to 3.5 with acetic acid (100), and add water to make 1000 mL. To 400 mL of this solution add 600 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of pivmecillinam is about 6.5 minutes.

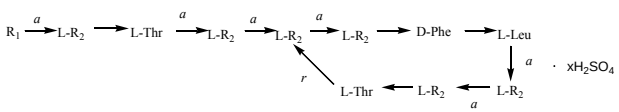
System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, pivmecillinam and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pivmecillinam to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Polymyxin B Sulfate



Polymyxin B_1 R_1 = 6-Methyloctanoic acid
R_2 = L-α-γ-Diaminobutyric acid

Polymyxin B_2 R_1 = 6-Methylheptanoic acid

R_2 = L-α-γ-Diaminobutyric acid
Thr: Threonine Phe: Phenylalanine
Leu: Leucine

N-{(2*S*)-4-Amino-1-{{(2*S*,3*R*)-1-{{(2*S*)-4-amino-1-oxo-1-{{(3*S*,6*S*,9*S*,12*S*,15*R*,18*R*,21*S*)-6,9,18-*tris*(2-aminoethyl)-15-benzyl-3-[(1*R*)-1-hydroxyethyl]-12-(2-methylpropyl)-2,5,8,11,14,17,20-heptaoxo-1,4,7,10,13,16,19-heptazacyclotricos-21-yl}amino}butan-2-yl}amino}-3-hydroxy-1-oxobutan-2-yl}amino}-1-oxobutan-2-yl}-6-methyloctanamide sulfate [*1405-20-5*]

Polymyxin B Sulfate is the sulfate of a mixture of peptide substances having antibacterial activity produced by the growth of *Bacillus polymyxa*.
Polymyxin B Sulfate contains not less than 6500 units (potency) per mg of polymyxin B ($C_{55\sim56}H_{96\sim98}N_{16}O_{13}$), calculated on the dried basis. One unit is equivalent to 0.129 μg of polymyxin B sulfate ($C_{55\sim56}H_{96\sim98}N_{16}O_{13}\cdot 1\sim2H_2SO_4$).

Description Polymyxin B Sulfate is a white to yellow-brown powder.
Polymyxin B Sulfate is freely soluble in water, and practically insoluble in ethanol (99.5).

Identification (1) To 5 mL of a solution of Polymyxin B Sulfate (1 in 10) add 5 mL of a solution of sodium hydroxide (1 in 10), and add 5 drops of a solution of copper (II) sulfate pentahydrate (1 in 100) while shaking: a purple color develops.

(2) Transfer 5 mg each of Polymyxin B Sulfate and Polymyxin B Sulfate RS into separate stoppered test tubes, dissolve in 1 mL of diluted hydrochloric acid (1 in 2), stopper the tubes, heat at 135 °C for 5 hours, evaporate to dryness in a water bath, and continue heating until the odor of hydrochloric acid is no longer perceptible. Dissolve the residues in 0.5 mL of water, and use these solutions as the test solution and standard solution (1). Separately, dissolve separately 20 mg each of L-leucine, L-threonine, phenylalanine, and L-serine in 10 mL of water, and use these solutions as the standard solutions (2), (3), (4), and (5), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 3 μL each of the test solution and standard solutions (1), (2), (3), (4), and (5) on a plate of silica gel for thin-layer chromatography. Expose the plate to a saturated vapor of the developing solvent for 15 hours, and develop the plate with a mixture of phenol and water (3 : 1) to a distance of about 13 cm without exposure to light. Dry the plate at 110 °C for 5 minutes, spray evenly ninhydrin-acetic acid TS on the plate, and heat at 110 °C for 5 minutes: the R_f value of each spot obtained from the test solution is the same as that of the corresponding spot obtained from the standard solution (1). Each spot obtained form the test solution appears at the position corresponding to the spots from the standard solutions (2), (3), and (4), but not at the position corresponding to the spot from the standard solution (5).

(3) A solution of Polymyxin B Sulfate (1 in 20) responds to the Qualitative Tests for sulfate.

Specific Optical Rotation $[\alpha]_D^{20}$: -78 ~ -90° (0.5 g calculated on the dried basis, water, 25 mL, 100 mm).

pH Dissolve 1.0 g of Polymyxin B Sulfate in 50 mL of water: the pH of this solution is between 5.0 and 7.0.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Polymyxin B Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Phenylalanine***—Weigh accurately 0.375 g of Polymyxin B Sulfate, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Determine the absorbances, A_1, A_2, A_3, A_4, and A_5 at 252 nm, 258 nm, 264 nm, 280 nm, and 300 nm of this solution as directed under Ultraviolet-visible Spectrophotometry. Calculate the amount of phenylalanine by the following equation: not less than 9.0 % and not more than 12.0 %.

$$\text{Amount (\%) of phenylalanine} = \frac{A_2 - 0.5A_1 + 0.5A_3 - 1.8A_4 + 0.8A_5}{W_T} \times 9.4787$$

W_T: Amount (g) of Polymyxin B Sulfate taken, calculated on the dried basis

Loss on Drying Not more than 6.0 % (1 g, in vacuum, 60 °C, 3 hours).

Residue on Ignition Not more than 0.75 % (1 g).

Sterility Test It meets the requirement, when Polymyxin B Sulfate is used in a sterile preparation.

Pyrogen Test It meets the requirement, when Polymyxin B Sulfate is used in a sterile preparation,. Weigh an appropriate amount of Polymyxin B Sulfate, dissolve in Isotonic Sodium Chloride Injection to make a solution so that each mL contains 20000 units, and use the solution as the test solution. The amount of injection is 1.0 mL of the test solution per kg of body weight of rabbit.

Assay ***The Cylinder-plate method*** (1) Agar media for seed and base layer-

Peptone	10.0g
Beef extract	3.0 g
Sodium chloride	30.0 g
Agar	20.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

(2) Test organism- *Escherichia coli* NIHJ.

(3) Weigh accurately an amount of Polymyxin B Sulfate, equivalent to about 200000 units (potency), dissolve in phosphate buffer solution, pH 6.0, to make exactly 20 mL. Pipet a suitable volume of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 4000 units (potency) and 1000 units (potency), and use these solutions as the high concentration test solution and low concentration test solution, respectively. Separately, weigh accurately about 200000 units (potency) of Polymyxin B Sulfate, and dissolve in phosphate buffer solution, pH 6.0, to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5 °C and use within 14 days. Pipet a suitable volume of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 4000 units (potency) and 1000 units (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively. Perform the test with these solutions according to the Cylinder-plate method (I 8) as directed under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Potassium Bromide

KBr: 119.00

[*7758-02-3*]

Potassium Bromide, when dried, contains not less than 99.0 % and not more than 101.0 % of potassium bromide (KBr).

Description Potassium Bromide appears as colorless or white crystals, granules or crystalline powder and is odorless.

Potassium Bromide is freely soluble in water or in glycerin, soluble in hot ethanol and slightly soluble in ethanol (95).

Identification A solution of Potassium Bromide (1 in 10) responds to the Qualitative Tests for potassium salt and for bromide.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Potassium Bromide in 3 mL of water: the solution is clear and colorless.

(2) ***Alkali***—Dissolve 1.0 g of Potassium Bromide in 10 mL of water, add 0.10 mL of 0.05 mol/L sulfuric acid VS and 1 drop of phenolphthalein TS, heat to boiling and cool: no color develops.

(3) ***Chloride***—Make a calculation from the result obtained in the Assay: not more than 84.5 mL of 0.1 mol/L silver nitrate VS is consumed for 1 g of Potassium Bromide.

(4) ***Sulfate***—Proceed with 2.0 g of Potassium Bromide and perform the test. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024 %).

(5) ***Iodide***—Dissolve 0.5 g of Potassium Bromide in 10 mL of water, add 2 to 3 drops of iron (III) chloride TS and 1 mL of chloroform and shake: no red-purple to purple color develops in the chloroform layer.

(6) ***Bromate***—Dissolve 1.0 g of Potassium Bromide in 10 mL of freshly boiled and cooled water and add 0.1 mL of potassium iodide TS, 1 mL of starch TS and 3 drops of dilute sulfuric acid. Shake the mixture gently and allow to stand for 5 minutes: no blue color develops.

(7) ***Heavy metals***—Proceed with 2.0 g of Potassium Bromide according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(8) ***Magnesium and alkaline-earth metals***—To 200 mL of water add 0.1 g of hydroxylammonium hydrochloride, 10 mL of pH 10 ammonium chloride buffer solution, 1 mL of 0.1 mol/L zinc sulfate VS, and 0.2 g of eriochrome black T-sodium chloride indicator, and warm at 40 °C. To this solution add dropwise 0.01 mol/L disodium ethylenediaminetetraacetate VS until until the red-purple color of the solution changes to blue-purple. To this solution add 10.0 g of Potassium Bromide dissolved in 100 mL of water. If the color changes to red-purple, titrate the solution with 0.01 mol/L disodium ethylenediaminetetraacetate VS until the color of the solution changes to blue-purple: not more than 5.0 mL of 0.01 mol/L disodium ethylenediaminetetraacetate is consumed (not more than 0.02 % as calcium).

(9) ***Barium***—Dissolve 0.5 g of Potassium Bromide in 10 mL of water, add 0.5 mL of dilute hydrochloric acid and 1 mL of potassium sulfate TS and allow to stand for 10 minutes: no turbidity is produced.

(10) ***Iron***—Dissolve 0.5 g of Potassium Bromide in water to make 10 mL and use this solution as the test solution. To 1 mL of standard iron solution add water to make 10 mL, and use this solution as the standard solution. To the test solution and standard solution add 2.0 mL of citric acid solution (1 in 5) and 0.1 mL of thioglycolic acid, alkalify to litmus with ammonia solution (28), and add water to make 20 mL. After 5 minutes, the color of the test solution is not more intense than that of the standard solution (not more than 20 ppm).

(11) ***Arsenic***—Prepare the test solution with 1.0 g of Potassium Bromide according to Method 1 and perform the test (not more than 2 ppm).

Loss on Drying Not more than 1.0 % (1 g, 110 °C, 4 hours).

Assay Weigh accurately about 0.4 g of Potassium Bromide, previously dried and dissolve in 50 mL of

water. Add 10 mL of dilute nitric acid and exactly measured 50 mL of 0.1 mol/L silver nitrate VS and titrate the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS
= 11.900 mg of KBr

Containers and Storage ***Containers***—Tight containers.

Potassium Chloride

KCl: 74.55

Potassium chloride [*7447-40-7*]

Potassium Chloride, when dried, contains not less than 99.0 % and not more than 101.0 % of potassium chloride (KCl).

Description Potassium Chloride appears as colorless or white crystals or crystalline powder, is odorless and has a saline taste.
Potassium Chloride is freely soluble in water, and practically insoluble in ethanol (95) or ether.
A solution of Potassium Chloride (1 in 10) is neutral.

Identification A solution of Potassium Chloride (1 in 50) responds to the Qualitative Tests for potassium salt and for chloride.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Potassium Chloride in 5 mL of water: the solution is clear and colorless.

(2) ***Acid and alkali***—Dissolve 5.0 g of Potassium Chloride in 50 mL of freshly boiled and cooled water and add 3 drops of phenolphthalein TS: no red color is observed. Then add 0.50 mL of 0.01 mol/L sodium hydroxide VS: a red color is observed.

(3) ***Bromide***—Dissolve 1.0 g of Potassium Chloride in water to make 100 mL. To 5 mL of the solution, add 3 drops of dilute hydrochloric acid and 1 mL of chloroform and add 3 drops of chloramine TS dropwise while shaking: no yellow to yellow-red color is observed in the chloroform layer.

(4) ***Iodide***—Dissolve 0.5 g of Potassium Chloride in 10 mL of water, add 3 drops of iron (III) chloride TS and 1 mL of chloroform, shake, allow to stand for 30 minutes and shake again: no red-purple to purple color is observed in the chloroform layer.

(5) ***Heavy metals***—Proceed with 4.0 g of Potassium Chloride according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 5 ppm).

(6) ***Sodium***—Dissolve 1.0 g of Potassium Chloride in 20 mL of water and perform the Flame Coloration Test (1): no persistent, yellow color is observed.

(7) ***Aluminum***—Perform this test when Potassium Chloride is used in preparations for hemodialylsis. Weigh accurately 2.0 g of Potassium Chloride, add 50 mL of water, and sonicate for 30 minutes. To this solution add 4 mL of nitric acid, add water to make 100 mL, and use this solution as the test solution. To a suitable amount of aluminum add 6 mol/L hydrochloric acid, and heat at 80 °C for a few minutes. Weigh accurately 100 mg of this aluminum, dissolve in a mixture of 10 mL of hydrochloric acid and 2 mL of nitric acid, and heat at 80 °C for about 30 minutes. Continue heating until the amount of the solution is reduced to about 4 mL, and cool to room temperature. To this solution add 4 mL of water, heat again to evaporate until the amount of the solution becomes 2 mL, cool, and add water to make 100 mL. Pipet 10.0 mL of this solution, and add water to make 100 mL. Pipet 1.0 mL of this solution, add water to make 100 mL and render the concentration of about 1.0 μg/mL, and use this solution as the standard solution. Perform the test with the test solution and standard solution as directed under Atomic Absorption Spectrophotometry according to the following conditions, and calculate the aluminum content of the test solution using the calibration curve obtained from the absorbances of the standard solution: not more than 1 ppm.

Lamp: Aluminum hollow cathode lamp
Wavelength: 309.3 nm
Blank solution: To 40 mL of nitric acid add water to make 1000 mL.

(8) ***Calcium and magnesium***—Dissolve 0.20 g of Potassium Chloride in 20 mL of water, add 2 mL of ammonia TS, 2 mL of ammonium oxalate TS and 2 mL of dibasic sodium phosphate TS and then allow to stand for 5 minutes: no turbidity is produced.

(9) ***Arsenic***—Prepare the test solution with 1.0 g of Potassium Chloride according to Method 1 and perform the test (not more than 2 ppm).

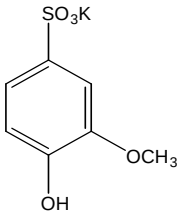
Loss on Drying Not more than 0.5 % (1 g, 130 °C, 2 hours).

Assay Weigh accurately about 0.2 g of Potassium Chloride, previously dried, dissolve in 50 mL of water and titrate with 0.1 mol/L silver nitrate VS while shaking vigorously (indicator: 1 mL of potassium chromate TS).

Each mL of 0.1 mol/L silver nitrate VS
= 7.455 mg of KCl

Containers and Storage ***Containers***—Tight containers.

Potassium Guaiacolsulfonate



Sulfogaiacol $C_7H_7KO_5S$: 242.29

Potassium 4-hydroxy-3-methoxybenzenesulfonate
[*1321-14-8*]

Potassium Guaiacolsulfonate contains not less than 98.5 % and not more than 101.0 % of potassium guaiacolsulfonate ($C_7H_7KO_5S$), calculated on the anhydrous basis.

Description Potassium Guaiacolsulfonate appears as crystals or crystalline powder and is odorless or has a slight, characteristic odor and a slightly bitter taste. Potassium Guaiacolsulfonate is freely soluble in water or in formic acid, slightly soluble in methanol and practically insoluble in ethanol, in ether or in acetic anhydride.

Identification (1) Take 10 mL of a solution of Potassium Guaiacolsulfonate (1 in 100) and add 2 drops of iron (III) chloride TS: a blue-purple color is observed.

(2) Dissolve separately 0.25 g of Potassium Guaiacolsulfonate and Potassium Guaiacolsulfonate RS in water to make 500 mL each, to 10 mL each of these solutions, add phosphate buffer solution, pH 7.0, to make 100 mL each and use these solution as the test solution and the standard solution, respectively. Determine the absorption spectra of the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) A solution of Potassium Guaiacolsulfonate (1 in 10) responds to Qualitative Tests for potassium salt.

pH Dissolve 1.0 g of Potassium Guaiacolsulfonate in 20 mL of water: the pH of the solution is between 4.0 and 5.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Potassium Guaiacolsulfonate in 20 mL of water: the solution is clear and colorless.

(2) ***Sulfate***—Perform the test with 0.8 g of Potassium Guaiacolsulfonate. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.030 %).

(3) ***Heavy metals***—Proceed with 1.0 g of Potassium Guaiacolsulfonate according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Arsenic***—Prepare the test solution with 1.0 g of Potassium Guaiacolsulfonate according to Method 1 and perform the test (not more than 2 ppm).

(5) ***Selenium***—Weigh 0.2 g of Potassium Guaiacolsulfonate and proceed as directed under the Oxygen Flask Combustion Method, using 25 mL of diluted nitric acid (1 in 30) as the absorbing liquid. Use a 1000 mL combustion flask. After combustion, wash the stopper and inside of the mask with 10 mL of water. Transfer the liquid inside the combustion flask to a 150 mL beaker using 20 mL of water, heat gently to boil and boil for 10 minutes. Cool to room temperature and use this solution as the test solution. Separately, pipet 6 mL of selenium standard stock solution, add 25 mL of diluted nitric acid (1 in 30) and 25 mL of water, and use this solution as the standard solution. To each of the test solution and the standard solution, add diluted ammonia solution (28) (1 in 2) to adjust the pH to 2.0 ± 0.2 and add water to dilute to exactly 60 mL. Transfer to a separatory funnel using 10 mL of water and wash the separatory funnel using 10 mL of water. Add 0.2 g of hydroxylamine and stir to dissolve. Add 5 mL of 2,3-diaminonaphthalene, insert a stopper, stir and allow to stand at room temperature for 100 minutes. Add 5.0 mL of cyclohexane, shake vigorously and allow to separate. Discard the aqueous layer, centrifuge the cyclohexane extract to remove the water content and take the cyclohexane layer. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry, using a solution prepared in the same manner with 25 mL of water added to 25 mL of diluted nitric acid (1 in 30), as the blank. Determine the absorbances at 380 nm: the absorbance of the solution obtained from the test solution is not more than the absorbance obtained from the standard solution (not more than 30 ppm).

(6) ***Related substances***—Dissolve 0.20 g of Potassium Guaiacolsulfonate in 200 mL of mobile phase and use this solution as the test solution. Pipet 1.0 mL of the test solution, add the mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform the test with 5μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area obtained from the test solution and the standard solution by the automatic integration method: the total area of peaks other than the peak of potassium guaiacolsulfonate from the test solution is not larger than the peak area of potassium guaiacolsulfonate from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (main wavelength: 279 nm).

Column: A stainless steel column, about 4 mm in internal diameter and 20 cm to 25 cm in length, packed with dimethylaminopropylsilanized silica gel (5 μm to 10 μm in particle diameter).

Column temperature: A constant temperature of about 30 °C.

Mobile phase: A mixture of 0.05 mol/L monobasic

potassium phosphate VS and methanol (20 : 1).

Flow rate: Adjust the flow rate so that the retention time of Potassium Guaiacolsulfonate is about 10 minutes.

System suitability

Detection sensitivity: Adjust the sensitivity so that the peak height of potassium guaiacolsulfonate from 5 μL of the standard solution is not less than 10 mm.

System performance: Weigh 50 mg each of Potassium Guaiacolsulfonate and guaiacol and dissolve in 50 mL of the mobile phase. When the procedure is run with 5 μL of this solution under the above operating conditions, guaiacol and potassium guaiacolsulfonate are eluted in this order with the resolution of their peaks being not less than 4.0.

Time span of measurement: About twice as long as the retention time of potassium guaiacolsulfonate.

Water 3.0 ~ 4.5 % (0.3 g, direct titration).

Assay Weigh accurately about 0.3 g of Potassium Guaiacolsulfonate, dissolve in 2.0 mL of formic acid, add 50 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 24.229 mg of $C_7H_7KO_5S$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Potassium Iodide

KI: 166.00

[*7681-11-0*]

Potassium Iodide, when dried, contains not less than 99.0 % and not more than 101.0 % of potassium iodide (KI).

Description Potassium Iodide appears as colorless or white crystals or a white crystalline powder.
Potassium Iodide is very soluble in water, soluble in ethanol (95) and practically insoluble in ether.
Potassium Iodide is slightly deliquescent in moist air.

Identification A solution of Potassium Iodide (1 in 20) responds to the Qualitative Tests for potassium salt and for iodide.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Potassium Iodide in 2 mL of water: the solution is clear and colorless.

(2) ***Alkali***—Dissolve 1.0 g of Potassium Iodide in 10 mL of freshly boiled and cooled water and add 0.50 mL of 0.005 mol/L sulfuric acid and 1 drop of phenolphthalein TS: no color is observed.

(3) ***Chloride, bromide and thiosulfate***—Dissolve 0.20 g of Potassium Iodide in 5 mL of ammonia TS, add 15.0 mL of 0.1 mol/L silver nitrate VS, shake for 2 minutes to 3 minutes and filter. To 10 mL of the filtrate, add 15 mL of dilute nitric acid: no brown color is observed. The solution has no more turbidity than that of the following control solution.

Control solution—Take 0.30 mL of 0.01 mol/L hydrochloric acid VS, add 2.5 mL of ammonia TS and 7.5 mL of 0.1 mol/L silver nitrate VS and 15 mL of dilute nitric acid.

(4) ***Nitrate, nitrite and ammonium***—Place 1.0 g of Potassium Iodide in a 40-mL test tube and add 5 mL of water, 5 mL of sodium hydroxide TS and 0.2 g of aluminum wire. Insert the absorbent cotton in the mouth of the test tube and place a piece of moistened red litmus paper on it. Heat the test tube carefully in a water-bath for 15 minutes: the gas evolved does not turn red litmus paper to blue.

(5) ***Cyanide***—Dissolve 0.5 g of Potassium Iodide in 10 mL of water. To 5 mL of this solution, add 1 drop of iron (II) sulfate TS and 2 mL of sodium hydroxide TS, warm and then add 4 mL of hydrochloric acid: no green color is observed.

(6) ***Iodate***—Dissolve 0.5 g of Potassium Iodide in 10 mL of freshly boiled and cooled water and add 2 drops of dilute sulfuric acid and 1 drop of starch TS: no blue color is observed immediately.

(7) ***Heavy metals***—Proceed with 2.0 g of Potassium Iodide according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(8) ***Sodium***—Dissolve 1.0 g of Potassium Iodide in 10 mL of water and perform the Flame Coloration Test (1): a yellow color is observed, but does not persist.

(9) ***Barium***—Dissolve 0.5 g of Potassium Iodide in 10 mL of water, add 1 mL of dilute sulfuric acid and allow to stand for 5 minutes: no turbidity is produced.

(10) ***Arsenic***—Prepare the test solution with 0.40 g of Potassium Iodide according to Method 1 and perform the test (not more than 5 ppm).

Loss on Drying Not more than 1.0 % (2 g, 105 °C, 4 hours).

Assay Weigh accurately about 0.5 g of Potassium Iodide, previously dried, in an iodine flask, dissolve in 10 mL of water, add 35 mL of hydrochloric acid and 5 mL of chloroform and titrate with 0.05 mol/L potassium iodate VS with shaking vigorously until the red-purple color of the chloroform layer disappears. The end point is reached when the red-purple color does not reappear in the chloroform layer within 5 minutes after the layer has been decolorized.

Each mL of 0.05 mol/L potassium iodate VS
= 16.600 mg of KI

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Potassium Permanganate

$KMnO_4$: 158.03

[*7722-64-7*]

Potassium Permanganate, when dried, contains not less than 99.0 % and not more than 101.0 % of potassium permanganate ($KMnO_4$).

Description Potassium Permanganate appears as deep purple crystals, has a metallic luster.
Potassium Permanganate is soluble in water.
A solution of Potassium Permanganate (1 in 1000) has a slightly sweet taste, astringent.

Identification A solution of Potassium Permanganate (1 in 100) responds to the Qualitative Tests for permanganate.

Purity (1) ***Water-insoluble substances***—Dissolve 2.0 g of Potassium Permanganate, previously powdered, in 200 mL of water. Filter the insoluble substances through a tared glass filter (G4), wash with water until the last washing shows no color and dry at 105 °C for 2 hours: the residue is not more than 4 mg.

(2) ***Arsenic***—Dissolve 0.40 g of Potassium Permanganate in 10 mL of water, add 1 mL of sulfuric acid, add a strong hydrogen peroxide solution until the solution remains colorless and evaporate on a sand-bath nearly to dryness. Dissolve the residue in 5 mL of water and perform the test with this solution as the test solution: the color produced is not more intense than the following standard color (not more than 5 ppm).

Standard color—To 10 mL of water, add 1 mL of sulfuric acid and the same volume of hydrogen peroxide solution (30) as used for the preparation of the test solution. Evaporate the solution on a sand-bath nearly to dryness, add 2.0 mL of standard arsenic solution and water to make 5 mL and perform the test with this solution in the same manner as the test solution.

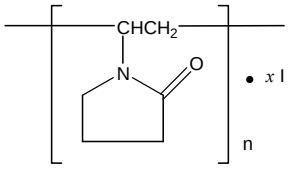
Loss on Drying Not more than 0.5 % (1 g, silica gel, 18 hours).

Assay Weigh accurately about 0.6 g of Potassium Permanganate, previously dried, dissolve in water to make exactly 200 mL and use this solution as the test solution. Pipet 25 mL of 0.05 mol/L oxalic acid VS into a Erlenmeyer flask, add 200 mL of diluted sulfuric acid (1 in 20) and keep at a temperature between 30 °C and 35 °C. Transfer the test solution to a buret. Add quickly 23 mL of the test solution from the buret to the flask while shaking gently and then allow the flask to stand until the red color disappears. Warm the mixture to a temperature between 55 °C and 60 °C and continue the titration slowly until the red color persists for 30 seconds.

Each mL of 0.05 mol/L oxalic acid VS
= 3.1607 mg of $KMnO_4$

Containers and Storage ***Containers***—Tight containers.

Povidone Iodine



$(C_6H_9NO)_n \cdot xI$

Poly(1-ethenylpyrrolidin-2-one)-iodine [*25655-41-8*]

Povidone Iodine is a complex of iodine with 1-vinyl-2-pyrrolidone polymer. Povidone Iodine contains not less than 9.0 % and not more than 12.0 % of available iodine (I: 126.90) and not less than 9.5 % and not more than 11.5 % of nitrogen (N: 14.01), calculated on the dried basis.

Description Povidone Iodine is a dark red-brown powder and has a characteristic odor.
Povidone Iodine is freely soluble in water or in ethanol (99.5).

pH—A solution of Povidone Iodine (1 in 100) is between 1.5 and 3.5.

Identification (1) To 10 mL of diluted starch TS (1 in 10), add 1 drop of a solution of Povidone Iodine (1 in 10): a deep blue color develops.

(2) To 1 mL of a solution of Povidone Iodine (1 in 100), add 1 mL of sodium thiosulfate TS and add 1 mL of ammonium thiocyanate-cobaltous nitrate TS and 2 drops of 1 mol/L hydrochloric acid TS: a blue color develops and a blue precipitate is gradually produced.

Purity (1) ***Clarity and color of solution***—Dissolve 0.30 g of Povidone Iodine in 100 mL of water: the solution is clear and brown.

(2) ***Heavy metals***—Proceed with 1.0 g of Povidone Iodine according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of

Povidone Iodine according to Method 4 and perform the test (not more than 2 ppm).

(4) ***Iodide ion***—Weigh 0.5 g of Povidone Iodine, dissolve in 100 mL of water and add sodium bisulfite TS until the color of iodine completely disappears. To this solution, add exactly 25 mL of 0.1 mol/L silver nitrate VS, shake well with 10 mL of nitric acid, titrate the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS until a red-brown color develops and calculate the total amount of iodine (indicator: 1 mL of ammonium iron (III) sulfate TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L ammonium thiocyanate VS
= 12.690 mg of I

Obtain the amount of iodide ion, calculated on the dried basis, by deducting the amount (%) of available iodine from the total amount (%) of iodine: it is not more than 6.6 %.

Loss on Drying Not more than 8.0 % (1 g, 100 ºC, 3 hours).

Residue on Ignition Not more than 0.05 % (5 g).

Assay (1) ***Available iodine***—Weigh accurately about 0.5 g of Povidone Iodine, dissolve in 30 mL of water and titrate with 0.02 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS).

Each mL of 0.02 mol/L sodium thiosulfate VS
= 2.5381 mg of I

(2) ***Nitrogen***—Weigh accurately about 1 g of Povidone Iodine, dissolve in water to make exactly 50 mL and use this solution as the test solution. Pipet 1.0 mL of the test solution and perform the test as directed under the Nitrogen Determination.

Each mL of 0.005 mol/L sulfuric acid VS
= 0.14007 mg of N

Containers and Storage ***Containers***—Tight containers.

Povidone Iodine Topical Solution

Povidone Iodine Topical Solution is a solution of povidone iodine for external use. Povidone Iodine Topical Solution contains not less than 85.0 % and not more than 120.0 % of the labeled amount of iodine (I: 126.90). Povidone Iodine Topical Solution may contain a small amount of ethanol.

Method of Preparation Prepare as directed under Solutions, with Povidone Iodine.

Identification (1) Take a portion of Povidone Iodine Topical Solution, equivalent to 50 mg of iodine according to the labeled amount, add water to make 100 mL. Pipet 1 mL of this solution and add 1 mL of starch TS and 9 mL of water: a deep blue color develops.

(2) Transfer 10 mL of Povidone Iodine Topical Solution to an Erlenmeyer flask, avoiding contact with the neck of the flask. Cover the mouth of the flask with a small disk of filter paper and wet it with 1 drop of starch TS: no blue color develops within 60 seconds.

Alcohol Content According to Method 2 under the Alcohol Number Determination Test, Povidone Iodine Topical Solution contains not less than 90.0 % and not more than 110.0 % of the labeled amount of ethanol.

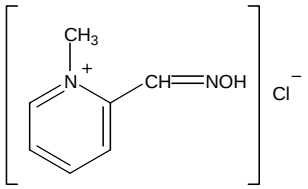
pH 1.5 ~ 6.5.

Assay Transfer an accurate volume of Povidone Iodine Topical Solution, equivalent to about 50 mg of iodine, to a glass-stoppered flask, add water to make 30 mL and titrate with 0.02 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination and make any necessary correction.

Each mL of 0.02 mol/L sodium thiosulfate VS
= 2.5380 mg of I

Containers and Storage ***Containers***—Tight containers.

Pralidoxime Chloride



$C_7H_9ClN_2O$: 172.61

[(*E*)-(1-Methylpyridin-2-ylidene)methyl]-oxoazanium chloride [*51-15-0*]

Pralidoxime Chloride contains not less than 97.0 % and not more than 102.0 % of pralidoxime chloride ($C_7H_9ClN_2O$), calculated on the dried basis.

Description Pralidoxime Chloride is a white and pale yellow crystalline powder and is odorless.
Pralidoxime Chloride is very soluble in water.
Pralidoxime Chloride is stable on the exposure to air.

Identification (1) The retention time of the principal peak from the test solution corresponds to that from the standard solution, under the Assay.

(2) Determine the infrared spectra of Pralidoxime

Chloride and Pralidoxime Chloride RS, previously dried, as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution (1 in 10) responds to the Qualitative Tests for chlorides.

Melting Point 215 ~ 225 °C (with decomposition).

Purity ***Heavy metals***—Proceed with 1.0 g of Pralidoxime Chloride according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

Loss on Drying Not more than 2.0 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.5 % (1 g).

Assay Transfer about 62.5 mg of Pralidoxime Chloride, accurately weighed, to a flask, dissolve in water, dilute with water to make 50 mL, mix and filter. Pipet 2 mL of the filtrate, add the mobile phase to make exactly 100 mL and use this solution as the test solution. Separately, dissolve a portion of Pralidoxime Chloride RS, accurately weighed, in water to obtain a known concentration of 1.25 mg per mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform the test with 15 μL each of the test solution and the standard solution, according the following operating conditions as directed under Liquid Chromatography. Measure the peak areas, A_T and A_S, of pralidoxime chloride for the test solution and the standard solution, respectivly.

$$\text{Amount (mg) of pralidoxime chloride } (C_7H_9ClN_2O) = 2.5 \times C \times \frac{A_T}{A_S}$$

C: Concentration (μg/mL) of pralidoxime chloride in the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (main wavelength: 270 nm).

Column: A stainless steel column, about 3 mm to 5 mm, in internal diameter and about 25 cm in length, packed with octadecylated silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of acetonitrile and tetraethyl ammonium chloride (52 : 48).

Flow rate: 1.2 mL/minute.

System suitability

System performnace: Dissolve 0.65 mg of pyridine-2-aldoxime in 1 mL of water. To 2 mL of this solution, add 2 mL of a solution of pralidoxime chloride RS containing 1.25 mg per mL and the mobile phase to make 100 mL. When the procedure is run with 15 μL of this solution according to the above operating conditions, pyridine-2-aldoxime and pralidoxime chloride are eluted in this order with the resolution between their peaks being not less than 4.0, and the number of theoretical plates and symmetry factor are not less than 4000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 6 times with 15 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of pralidoxime is not more than 2.0 %.

Tetraethylammonium chloride solution—Dilute 3.4 mL of phosphoric acid (10 in 100), dissolve 0.17 g of tetraethylammonium chloride, add water to make 1000 mL.

Containers and Storage ***Containers***—Well-closed containers.

Pralidoxime Chloride Tablets

Pralidoxime Chloride Tablets contain not less than 95.0 % and not more than 105.0 % of the labeled amount of pralidoxime chloride ($C_7H_9ClN_2O \cdot HCl$: 172.61).

Method of Preparation Prepare as directed under Tablets, with Pralidoxime Chloride.

Identification The retention time of the principal peak from the test solution corresponds to that from the standard solution, under the Assay.

Dissolution Test Perform the test with 1 tablet of Pralidoxime Chloride Tablets at 100 revolutions per minute according to Method 1 under the Dissolution Test, using 900 mL of water as the dissolution solution. Take the dissolved solution after 60 minutes from start of the test, filter, dilute with the water, if necessary and use the solution as the test solution. Separately, weigh accurately a portion of Pralidoxime Chloride RS, dilute with the water to make the same concentration as the test solution and use this solution as the standard solution. Perform the test with test solution and standard solution, using water as the blank, to determin the absorbances at the wavelength of a maximum absorption near 293 nm as directed under Ultraviolet-visible Spectrophtometry.

The dissolution rate of Pralidoxime Chloride Tablets in 60 minutes is not less than 55 %.

Uniformity of Dosage Units It meets the requirement.

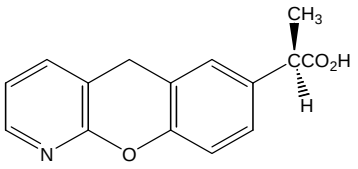
Assay Weigh accurately not less than 20 Pralidoxime Chloride Tablets and finely powder. Take an accurately weighed portion of the powder, equivalent to about 0.25 g of pralidoxime chloride, add 150 mL of water and mechanically swirl for 30 minutes. Add water to

make exactly 200 mL, centrifuge, pipet 2 mL of the clear supernatant liquid and add the mobile phase to make exactly 100 mL and use this solution as the test solution. Separately, weigh accurately about 50 mg of Pralidoxime Chloride RS and dissolve with water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL and use this solution as the standard solution. Proceed with the test solution and the standard solution as directed in the Assay under Pralidoxime Chloride.

$$\text{Amount (mg) of pralidoxime chloride } (C_7H_9ClN_2O) = \text{Amount (mg) of Pralidoxime Chloride RS} \times \frac{A_T}{A_S} \times 5$$

Containers and Storage ***Containers***—Well-closed containers.

Pranoprofen



and enantiomer

$C_{15}H_{13}NO_3$: 255.27

2-(5*H*-Chromeno[2,3-b]pyridin-7-yl)propanoic acid
[*52549-17-4*]

Pranoprofen, when dried, contains not less than 98.5 % and not less than 101.0 % of pranoprofen ($C_{15}H_{13}NO_3$).

Description Pranoprofen is a white to pale yellowish white crystalline powder.
Pranoprofen is freely soluble in *N,N*-dimethylformamide, soluble in acetic acid (100), sparingly soluble in methanol, slightly soluble in acetonitrile, in ethanol (95) or in acetic anhydride, very slightly soluble in ether and practically insoluble in water.
A solution of Pranoprofen in *N,N*-dimethylformamide (1 in 30) shows no optical rotation.

Identification (1) Dissolve 20 mg of Pranoprofen and Pranoprofen RS in 1 mol/L hydrochloric acid TS to make 100 mL. Pipet 10 mL each of these solutions and add water to make 100 mL. Determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry using 0.1 mol/L hydrochloric acid TS as the blank: both spectra exhibit similar intensities of absorption at the same wavelength.

(2) Determine the infrared spectra of Pranoprofen and Pranoprofen RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 186 ~ 190 ºC.

Purity (1) ***Chloride***—Dissolve 0.5 g of Pranoprofen in 40 mL of methanol and 6 mL of dilute nitric acid and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.30 mL of 0.01 mol/L hydrochloric acid, add 40 mL of methanol, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.021 %).

(2) ***Heavy metals***—Proceed with 2.0 g of Pranoprofen according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of the standard lead solution (not more than 10 ppm).

(3) ***Related Substances***—Dissolve 50 mg of Pranoprofen in 50 mL of the mobile phase and use this solution as the test solution. Pipet 1 mL of the test solution, add the mobile phase to make exactly 200 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine each peak area from both solutions by the automatic integration method: the each area of the peaks other than the principal peak from the test solution is not larger than peak area of the principal peak from the standard solution and the total peak area of them is not larger than twice of the peak area of the principal peak from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 275 nm).

Column: A stainless steel column, about 6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 ºC.

Mobile phase: Dissolve 7.02 g of sodium perchlorate in 1000 mL of water and adjust the pH to 2.5 with perchloric acid. To 2 volumes of this solution, add 1 volume of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of pranoprofen is about 10 minutes.

System suitability

Detection sensitivity: Adjust the detection sensitivity so that the peak height of pranoprofen from 10 μL of the standard solution is between 10 mm and 20 mm.

System performance: Dissolve 4 mg each of Pranoprofen and ethyl parahydroxybenzoate in 200 mL of the mobile phase. When the procedure is run with 10 μL of this solution according to the above operating conditions pranoprofen and ethyl parahydroxybenzoate are eluted in this order with the resolution between their peaks being not less than 2.1.

Time span of measurement: About 3 times as long as the retention time of pranoprofen.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, P_2O_5, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

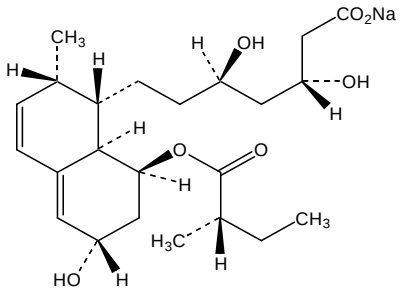
Assay Weigh accurately about 0.4 g of Pranoprofen, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 25.527 mg of $C_{15}H_{13}NO_3$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Pravastatin Sodium



$C_{23}H_{35}NaO_7$: 446.51

Sodium(3*R*,5*R*)-3,5-dihydroxy-7-((1*S*,2*S*,6*S*,8*S*,8a*R*)-6-hydroxy-2-methyl-8-(((*S*)-2-methylbutanoyl)oxy)-1,2,6,7,8,8a-hexahydro-naphthalen-1-yl)heptanoate
[*81131-70-6*]

Pravastatin Sodium contains not less than 98.5 % and not more than 101.0 % of pravastatin sodium ($C_{23}H_{35}NaO_7$), calculated on the anhydrous and solvent-free basis.

Description Pravastatin Sodium is a white to yellowish white, powder or crystalline powder.
Pravastatin Sodium is freely soluble in water or in methanol, and soluble in ethanol (99.5).
Pravastatin Sodium is hygroscopic.

Identification (1) Determine the absorption spectra of solutions of Pravastatin Sodium and Pravastatin Sodium RS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectrum of Pravastatin Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry: it exhibits absorption at the wavenumbers of about 2970 cm^{-1}, 2880 cm^{-1}, 1727 cm^{-1} and 1578 cm^{-1}.

(3) A solution of Pravastatin Sodium (1 in 10) responds to the Qualitative Tests (1) for sodium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: +153 ~ +159º (0.1 g calculated on the anhydrous and solvent-free basis, water, 20 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g of Pravastatin Sodium in 20 mL of freshly boiled and cooled water is between 7.2 and 8.2.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Pravastatin Sodium according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) ***Related substances***—Dissolve 0.10 g of Pravastatin Sodium in 100 mL of a mixture of water and methanol (11 : 9), and use this solution as the test solution. Pipet 10 mL of the test solution, add the mixture of water and methanol (11 : 9) to make exactly 100 mL. Pipet 5 mL of this solution, add the mixture of water arid methanol (11:9) to make exactly 100 mL, and use this solution as the standard solution. Perfom the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than pravastatin form the test solution is not larger than 0.2 times the peak area of pravastatin from the standard solution, and the total area of the peaks other than pravastatin form the test solution is not larger than the peak area of pravastatin from the standard solution. Keep the test solution and the standard solution at not over than 15 °C.

Operating condition

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability

Test for required detectability: To exactly 5 mL of the standard solution add a mixture of water and methanol (11 : 9) to make exactly 50 mL. Confirm that the peak area of pravastatin obtained with 10 μL of this solution is equivalent to 7 % to 13 % of that with 10 μL of the standard solution.

System performance: Dissolve 5 mg of Pravastatin Sodium in 50 mL of the mixture of water and methanol (11 : 9). When the procedure is run with 10 μL of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of pravastatin are not less than 3500 and not more than 1 .6, respectively.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pravastatin is not more than 2.0 %.

Time span of measurement: About 2.5 times as long as the retention time of pravastatin beginning after the solvent peak.

Water Not more than 4.0 % (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.1 g of Pravastatin Sodium and dissolve in a mixture of water and methanol (11 : 9) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and the mixture of water and methanol (11 : 9) to make exactly 100 mL and use this solution as the test solution. Separately, weigh accurately about 30 mg of Pravastatin 1,1,3,3-Tetramethylbutylamine RS (previously determine the water with 0.5 g by direct titration in volumetric titration), dissolve in the mixture of water and methanol (1 1 : 9) to make exactly 25 mL. Proceed with exactly 10 μL of this solution in the same manner for the preparation of the test solution, and use the solution so obtained as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of pravastatin to that of the internal standard.

$$\text{Amount (mg) of pravastatin sodium } (C_{23}H_{35}NaO_7) = W_S \times \frac{Q_T}{Q_S} \times 4 \times 1.0518$$

W_S: Amount (mg) of pravastatin in the amount of Pravastatin 1,1,3,3-Tetramethyl-butylamine RS taken, calculated on the anhydrous basis

Internal sandard solution—A solution of ethyl parahydroxybenoate in the mixture of water and methanol (11 : 9) (3 in 4000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 238 tim).

Column: A stainless steel column 4.6 mm in internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: A mixture of water, methanol, acetic acid (100) and triethylamine (550 : 450 : 1 : 1).

Flow rate: Adjust the flow rate so that the retention time of pravastatin is about 21 minutes.

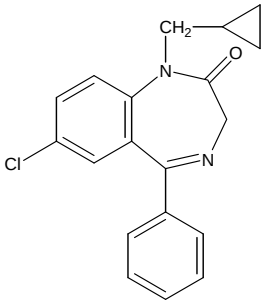
System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating condition, the internal standard and pravastatin are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with l0 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pravastatin to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Prazepam



$C_{19}H_{17}ClN_2O$: 324.80

7-Chloro-1-(cyclopropylmethyl)-5-phenyl-1*H*-benzo[e][1,4]diazepin-2(3*H*)-one [*2955-38-6*]

Prazepam, when dried, contains not less than 98.5 % and not more than 101.0 % of prazepam ($C_{19}H_{17}ClN_2O$).

Description Prazepam appears as white to pale yellow crystals or crystalline powder and is odorless.
Prazepam is freely soluble in acetone, soluble in acetic anhydride, sparingly soluble in dehydrated ethanol or in ether and practically insoluble in water.

Identification (1) Dissolve 10 mg of Prazepam in 3 mL of sulfuric acid and observe under ultraviolet light (main wavelength: 365 nm): the solution shows a grayish blue fluorescence.

(2) Dissolve 10 mg each of Prazepam and Prazepam RS in 1000 mL of a solution of sulfuric acid in dehydrated ethanol (3 in 1000) and determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Prazepam and Prazepam RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(4) Perform the Flame Coloration Test (2) with Prazepam: a green color develops.

Melting Point 145 ~ 148 ºC.

Purity (1) ***Chloride***—Take 1.0 g of Prazepam, add 50 mL of water, allow to stand for 1 hour with occasional shaking and filter. To 20 mL of the filtrate, add 6

mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 40 mL of 0.0l mol/L hydrochloric acid VS (not more than 0.036 %).

(2) ***Sulfate***—To 20 mL of the filtrate obtained in (1), add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048 %).

(3) ***Heavy metals***—Proceed with 2.0 g of Prazepam according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(4) ***Arsenic***—Prepare the test solution with 1.0 g of Prazepam according to Method 3 and perform the test (not more than 2 ppm).

(5) ***Related substances***—Dissolve 0.40 g of Prazepam in 10 mL of acetone and use this solution as the test solution. Pipet 1 mL of the test solution and add acetone to make exactly 20 mL. Pipet 1 mL of this solution, add acetone to make exactly 25 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and acetone (9 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.2 % (1 g, 105 ºC, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.4 g of Prazepam, previously dried, dissolve in 60 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.480 mg of $C_{19}H_{17}ClN_2O$

Containers and Storage ***Containers***—Tight containers.

Prazepam Tablets

Prazepam Tablets contain not less than 93.0 % and not more than 107.0 % of the labeled amount of prazepam ($C_{19}H_{17}ClN_2O$: 324.80).

Method of Preparation Prepare as directed under the Tablets, with Prazepam.

Identification (1) Take a portion of powdered Prazepam Tablets, equivalent to 50 mg of Prazepam according to the labeled amount, add 25 mL of acetone, shake well and filter. Take 5 mL of the filtrate, evaporate on a water-bath to dryness and dissolve the residue in 3 mL of sulfuric acid. With this solution, proceed as directed in the Identification (1) under Prazepam.

(2) Take a portion of powdered Prazepam Tablets, equivalent to 20 mg of prazepam according to the labeled amount, add 200 mL of a solution of sulfuric acid in dehydrated ethanol (3 in 1000), shake well and filter. To 5 mL of the filtrate and add a solution of sulfuric acid in dehydrated ethanol (3 in 1000) to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 241 nm and 245 nm, between 283 nm and 287 nm and between 363 nm and 367 nm and minima between 263 nm and 267 nm and between 334 nm and 338 nm.

Dissolution Test Proceed with 1 tablet of Prazepam Tablets according to Method 1 in the Dissolution Test and perform the test, using 900 mL of 0.1 mol/L hydrochloric acid TS as the dissolution solution at 100 rotations per minute. After 30 minutes from the start of the test, separate 20 mL or more of the dissolved solution and filter with a membrane filter with a pore size not more than 0.8 μm. Discard the first 10 mL of the filtrate, measure exactly the subsequent V mL of the filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly V' mL so that each mL of this solution might contain about 5 μg of Prazepam ($C_{19}H_{17}ClN_2O$) according to the labeled amount and use this solution as the test solution. Separately, weigh accurately about 5 mg of Prazepam RS, previously dried at 105 ºC for 2 hours, add 200 mL of 0.1 mol/L hydrochloric acid TS and dissolve with shaking, or by ultrasonication, if necessary, add 0.1 mol/L hydrochloric acid TS to make exactly 1000 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 240 nm as directed under Ultraviolet-visible Spectrophotometry.
The dissolution rate of Prazepam Tablets in 30 minutes is not less than 80 %.

$$\text{Dissolution rate (\%) with respect to the labeled amount of prazepam } (C_{19}H_{17}ClN_2O) = W_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{90}{C}$$

W_S: Amount (mg) of Prazepam RS,
C: Labeled amount (mg) of prazepam ($C_{19}H_{17}ClN_2O$) in 1 tablet.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Prazepam Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of prazepam ($C_{19}H_{17}ClN_2O$), add 30 mL of acetone, shake well, centrifuge and separate the supernatant. Repeat the same procedure twice with 30 mL each of acetone, combine all the supernatants and evaporate on a water-bath to dryness. Dissolve the residue in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.02 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.02 mol/L perchloric acid VS
= 6.496 mg of $C_{19}H_{17}ClN_2O$

Containers and Storage ***Containers***—Tight containers.

Precipitated Calcium Carbonate

$CaCO_3$: 100.09

[*471-34-1*]

Precipitated Calcium Carbonate, when dried, contains not less than 98.5 % and not more than 101.0 % of calcium carbonate ($CaCO_3$).

Description Precipitated Calcium Carbonate is a white, fine crystalline powder, is odorless and tasteless.
Precipitated Calcium Carbonate is practically insoluble in water (but its solubility in water increased in the presence of carbon dioxide),and practically insoluble in ethanol (95) or in ether.
Precipitated Calcium Carbonate dissolves with effervescence in dilute acetic acid, in dilute hydrochloric acid or in dilute nitric acid.

Identification (1) Dissolve 0.5 g of Precipitated Calcium Carbonate in 10 mL of dilute hydrochloric acid, boil, then cool and neutralize with ammonia TS: the solution responds to the Qualitative Tests for calcium salt.

(2) Precipitated Calcium Carbonate responds to the Qualitative Tests (1) for carbonate.

Purity (1) ***Acid-insoluble substances***—To 5.0 g of Precipitated Calcium Carbonate, add 50 mL of water, then add 20 mL of hydrochloric acid drop-wise with stirring, boil for 5 minutes, cool, add water to make 200 mL and filter through filter paper for Assay. Wash the residue until the last washing shows no turbidity with silver nitrate TS and ignite the residue together with the filter paper: the weight of the residue is not more than 10.0 mg.

(2) ***Heavy metals***—Mix 2.0 g of Precipitated Calcium Carbonate with 5 mL of water, add slowly 6 mL of dilute hydrochloric acid and evaporate on a water-bath to dryness. Dissolve the residue in 50 mL of water and filter. To 25 mL of the filtrate, add 2 mL of dilute acetic acid, 1 drop of ammonia TS and water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 3 mL of hydrochloric acid on a water-bath to dryness and add 2 mL of dilute acetic acid, 2.0 mL of standard lead solution and water to make 50 mL (not more than 20 ppm).

(3) ***Lead***—Weigh accurately 1.0 g of Precipitated Calcium Carbonate, mix with 5 mL of water, and add slowly 8 mL of 3 mol/L hydrochloric acid TS. Evaporate to dryness in a steam bath, dissolve the residue in 5 mL of water, and use this solution as the test solution. Transfer the test solution to a separatory funnel, wash with 10 mL of water, add 6 mL of a solution of diammonium hydrogen citrate, 2 mL of hydroxylamine hydrochloride TS and 2 drops of phenol red TS, and alkalify with ammonia solution (28). Cool the solution if necessary, add 2 mL of a solution of potassium cyanide, extract with 5 mL volumes of dithizone solution for extraction until the extracts show a green color, and combine the extracts in another separatory funnel. To the combined extracts add 20 mL of diluted nitric acid (1 in 1000), shake for 30 seconds, and discard the chloroform layer. To the nitric acid layer add 5.0 mL of standard dithizone solution and 4 mL of ammonia-cyanide TS, and shake for 30 seconds: the purple color of the chloroform layer is not more intense than the color obtained by proceeding with 3 mL of diluted standard lead solution (1 in 10) in the same manner as the test solution (not more than 3 ppm).

(4) ***Magnesium and alkali metals***—Dissolve 1.0 g of Precipitated Calcium Carbonate in a mixture of 20 mL of water and 10 mL of dilute hydrochloric acid, boil, neutralize with ammonia TS and add ammonium oxalate TS until precipitation of calcium oxalate is completed. Heat the mixture in a water-bath for 1 hour, cool, dilute with water to make 100 mL, shake well and filter. To 50 mL of the filtrate, add 0.5 mL of sulfuric acid, evaporate to dryness and ignite at 600 °C to a constant mass: the weight of the residue is not more than 5.0 mg.

(5) ***Barium***—Mix 1.0 g of Precipitated Calcium Carbonate with 10 mL of water, add drop-wise 4 mL of hydrochloric acid with stirring, boil for 5 minutes, cool, and add water to make 40 mL and filter. With the filtrate, perform the test as directed under Flame Coloration Test (1): no green color appears.

(6) ***Iron***—Weigh accurately about 40 mg of Precipitated Calcium Carbonate, dissolve in 5 mL of 2 mol/L hydrochloric acid TS, transfer to a beaker, add water to make 10 mL, and use this solution as the test solution. Separately, put 4.0 mL of standard iron solution into a beaker, add water to make 10 mL, and use this solution as the standard solution. To each beaker add 2 mL of a solution of citric acid (1 in 5) and two drops of thioglycolic acid, adjust the pH to 9.5 ± 0.1 with ammonia TS, add water to make 20 mL, mix, and

allow to stand for 5 minutes. Add water to make 50 mL, and mix well. Immediately determine the absorbances at 530 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry, using water as the blank: the absorbance of the test solution is not more than that of the standard solution (not more than 0.1 %).

(7) ***Arsenic***—Moisten 0.67 g of Precipitated Calcium Carbonate with 1 mL of water, then dissolve in 4 mL of dilute hydrochloric acid, use this solution as the test solution and perform the test (not more than 3 ppm).

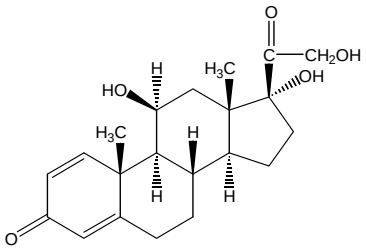
Loss on Drying Not more than 1.0 % (1 g, 180 °C, 4 hours).

Assay Weigh accurately about 0.12 g of Precipitated Calcium Carbonate, previously dried and dissolve in 20 mL of water and 3 mL of dilute hydrochloric acid. Add 80 mL of water, 15 mL of a solution of potassium hydroxide (1 in 10) and 50 mg of NN indicator and titrate immediately with 0.05 mol/L disodium ethylenediaminetetraacetate VS until the color of the solution changes from red-purple to blue.

Each mL of 0.05 mol/L
disodium ethylenediaminetetraacetate VS
= 5.004 mg of $CaCO_3$

Containers and Storage ***Containers***—Tight containers.

Prednisolone



$C_{21}H_{28}O_5$: 360.44

(8*S*,9*S*,10*R*,11*S*,13*S*,14*S*,17*R*)-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13-dimethyl-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[a]phenanthren-3-one [*50-24-8*]

Prednisolone, when dried, contains not less than 97.0 % and not more than 102.0 % of prednisolone ($C_{21}H_{28}O_5$).

Description Prednisolone is a white, crystalline powder and is odorless.
Prednisolone is soluble in methanol or in ethanol (95), slightly soluble in ethyl acetate or in chloroform and very slightly soluble in water.

Melting point—About 235 ºC (with decomposition).

Identification (1) Take 2 mg of Prednisolone, add 2 mL of sulfuric acid and allow to stand for 2 to 3 minutes: a deep red color, without fluorescence, develops. To this solution, add 10 mL of water cautiously: the color disappears and a gray, flocculent precipitate is produced.

(2) Determine the infrared spectra of Prednisolone and Prednisolone RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears, dissolve each in ethyl acetate, evaporate to dryness and repeat the test on the residues.

Specific Optical Rotation $[\alpha]_D^{20}$: +113 ~ +119º (after drying, 0.2g, ethanol (95), 20 mL, 100 mm).

Purity (1) ***Selenium***—To about 0.10 g of Prednisolone add 0.5 mL of a mixture of perchloric acid and sulfuric acd (1 : 1) and 2 mL of nitric acid and heat on a water bath until no more brown gas evolves and the solution becomes to be a pale yellow clear solution. After cooling, add 4 mL of nitric acid to this solution, then add water to make exactly 50 mL and use this solution as the test solution. Separatly, pipet 3 mL of selenium standard solution, add 0.5 mL of a mixture of perchloric acid and sulfuric acid (1 : 1) and 6 mL of nitric acid, then add water to make exactly 50 mL and use this solution as the stndard solution. Perform the test with the test solution and the standard solution as directed under the Atomic Absorption Spectrophotometry according to the following operating conditions and determine constant absorbances, A_T and A_S, obtained on a recorder after rapid increasing of the absorption: A_T is smaller than A_S (not more than 30 ppm). Perform the test by using a hydride generating system and a thermal absorption cell.

Lamp: A selenium hollow cathode lamp
Wavelength: 196.0 nm
Temperature of sample atomizer: About 1000 ºC, when an electric furnace is used.
Carrier gas: Nitorgen or argon.

(2) ***Related substances***—Dissolve about 20 mg of Prednisolone, dissolve in exactly 2 mL of a mixture of methanol and chloroform (1 : 1) and use this solution as the test solution. Separately, dissolve 20 mg of Hydrocortisone RS and 10 mg of Prednisolone Acetate RS each in a mixture of methanol and chloroform (1 : 1) to make exactly 100 mL and use these solutions as the standard solution (1) and the standard solution (2). Perform the test with the test solution and the standard solutions (1) and (2) as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution, the standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, toluene and diethylamine (55 : 45 : 2) to a distance of about 15 cm and air-dry

the plate (do not dip the filter paper in the developing vessel). Spray evenly alkaline blue tetrazolium TS on the plate: the spots from the test solution corresponding to those from the standard solutions (1) and (2) are not more intense than the spots from the standard solutions (1) and (2) and no spots other than the principal spot, hydrocortisone and prednisolone acetate appear from the test solution.

Loss on Drying Not more than 1.0 % (0.5 g, 105 ºC, 3 hours).

Residue on Ignition Not more than 0.1 % (0.5 g).

Assay Dissolve about 25 mg each of Prednisolone and Prednisolone RS, previously dried and accurately weighed, in 50 mL of methanol, add 25.0 mL of the internal standard solution to each and add methanol to make exactly 100 mL. To 1.0 mL each of these solution add the mobile phase to make exactly 10 mL and use these solutions as the test solution and the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and calculate the ratios, Q_T and Q_S, of the peak area of prednisolone to that of the internal standard, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of prednisolone}(C_{21}H_{28}O_5) = \text{Amount (mg) of Prednisolone RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (1 in 2000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 247 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with fluorosilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 ºC.

Mobile phase: A mixture of water and methanol (13 : 7).

Flow rate: Adjust the flow rate so that the retention time of prednisolone is about 15 minutes.

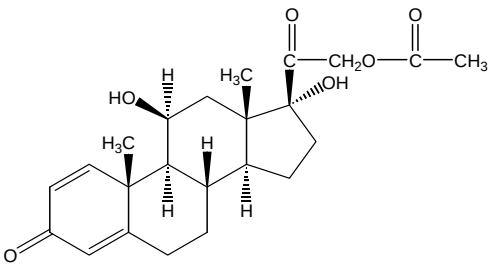
System suitability

System performance: Dissolve 25 mg of Prednisolone and 25 mg of hydrocortisone in 100 mL of methanol. To 1 mL of this solution add the mobile phase to make 10 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, hydrocortisone and prednisolone are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of prednesolone to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Prednisolone Acetate



$C_{23}H_{30}O_6$: 402.48

2-((8*S*,9*S*,10*R*,11*S*,13*S*,14*S*,17*R*)-11,17-Dihydroxy-10,13-dimethyl-3-oxo-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[a]phenanthren-17-yl)-2-oxoethyl acetate [*52-21-1*]

Prednisolone Acetate, when dried, contains not less than 96.0 % and not more than 102.0 % of prednisolone acetate ($C_{23}H_{30}O_6$).

Description Prednisolone Acetate is a white, crystalline powder.

Prednisolone Acetate is slightly soluble in methanol, in ethanol (95), in ethanol (99.5) or in chloroform and practically insoluble in water.

Melting point—About 235 °C (with decomposition).

Identification (1) Take 2 mg of Prednisolone, add 2 mL of sulfuric acid and allow to stand for 2 to 3 minutes: a deep red color, without fluorescence, develops. To this solution, add 10 mL of water cautiously: the color disappears and a gray, flocculent precipitate is produced.

(2) Determine the infrared spectra of Prednisolone Acetate and Prednisolone Acetate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears, dissolve each in ethanol (99.5), evaporate the anhydrous ethanol to dryness and repeat the test on the residues.

Specific Optical Rotation $[\alpha]_D^{20}$: +128 ~ +137° (after drying, 70 mg, methanol, 20 mL, 100 mm).

Purity ***Related substances***—Dissolve 0.20 g of Prednisolone Acetate in exactly 10 mL of a mixture of chloroform and methanol (9 : 1) and use this solution

as the test solution. Separately, dissolve 20 mg each of prednisolone, cortisone acetate and hydrocortisone acetate in a mixture of chloroform and methanol (9 : 1) to make exactly 10 mL and use this solution as the standard solution. Perform the test with the the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, ether, methanol and water (385 : 75 : 40 : 6) to a distance of about 15 cm and air-dry the plate. Examine under ultraviolet light (wavelength: 254 mm): the spots from the test solution corresponding to those from the standard solution are not more intense than the spots from the standard solution and any spot other than the principal spot, prednisolone, cortisone acetate and hydrocortisone acetate from the test solution do not appear.

Loss on Drying Not more than 1.0 % (0.5 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (0.5 g).

Assay Dissolve about 10 mg each of Prednisolone Acetate and Prednisolone Acetate RS, previously dried and accurately weighed, in 60 mL each of methanol, add exactly 2 mL each of the internal standard solution, then add methanol to make 100 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak height of prednisolone acetate to that of the internal standard, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of prednisolone acetate } (C_{23}H_{30}O_6) = \text{Amount (mg) of Prednisolone Acetate RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of butyl *p*-hydrosybenzoate in methanol (3 in 1000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: A mixture of water and acetonitrile (3 : 2).

Flow rate: Adjust the flow rate so that the retention time of prednisolone acetate is about 10 minutes.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, prednisolone acetate and the internal standard are eluted in this order with the resolution between their peaks being not less than 10.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of prednisolone acetate to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Prednisolone Sodium Succinate for Injection

Prednisolone Sodium Succinate for Injection is a preparation for injection which is reconstituted before use. Prednisolone Sodium Succinate for Injection contains not less than 72.4 % and not more than 83.2 % of prednisolone sodium succinate ($C_{25}H_{31}NaO_8$: 482.51) and the equivalent of not less than 90.0 % and not more than 110.0 % of the labeled amount of prednisolone ($C_{21}H_{28}O_5$: 360.44). The amount of Prednisolone Sodium Succinate for Injection is labeled as the amount of prednisolone ($C_{21}H_{28}O_5$).

Method of Preparation Prepare as directed under Injections, with Prednisolone Succinate and Dried Sodium Carbonate or Sodium Hydroxide. Prednisolone Sodium Succinate for Injection contains a suitable buffering agent.

Description Prednisolone Sodium Succinate for Injection appears as white powder or porous, friable mass. Prednisolone Sodium Succinate for Injection is freely soluble in water.
Prednisolone Sodium Succinate for Injection is hygroscopic.

Identification (1) Take 2 mg of Prednisolone Sodium Succinate for Injection add 2 mL of sulfuric acid and allow to stand for 2 to 3 minutes: a deep red color, without fluorescence, develops. To this solution add 10 mL of water cautiously: the color disappears and a gray, flocculent precipitate is formed.

(2) Dissolve 10 mg of Prednisolone Sodium Succinate for Injection in 1 mL methanol and add Fehling's TS and heat: an orange to red precipitate is formed.

(3) Dissolve 0.1 g of Prednisoione Sodium Succinate for Injection in 2 mL of sodium hydroxide TS, allow to stand for 10 minutes and filter. Add 1 mL of dilute hydrochloric acid to the shake, filtrate, if necessary. Adjust the solution with diluted ammonia TS (1 in 10) to a pH of about 6 and add 2 to 3 drops of iron (III)

chloride TS: a brown precipitate is formed.

(4) Prednisolone Sodium Succinate for Injection responds to the Qualitative Tests (1) for sodium salt.

pH Dissolve 1.0 g of Prednisolone Sodium Succinate for Injection in 40 mL of water: the pH of the solution is between 6.5 and 7.2.

Purity ***Clarity and color of solution***—Dissolve 0.25 g of Prednisolone Sodium Succinate for Injection in 10 mL of water: the solution is clear and colorless.

Loss on Drying Not more than 2.0 % (0.15 g, in vacuum, P_2O_5, 60 ºC, 3 hours).

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 2.4 EU/mg of prednisolone.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Take a number of sealed containers of Prednisolone Sodium Succinate for Injection, equivalent to about 0.10 g of prednisolone ($C_{21}H_{28}O_5$) and dissolve the contents in a suitable amount of diluted methanol (1 in 2) and transfer to a volumetric flask. Wash each container with diluted methanol (1 in 2), collect the washings in the volumetric flask and add diluted methanol (1 in 2) to make exactly 100 mL. Pipet 4 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, mix by shaking and use this solution as the test solution. Separately, weigh accurately about 25 mg of Prednisolone Succinate RS, previously dried in a desiccator for 6 hours (in vacuum, P_2O_5, 60 ºC), dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution. add diluted methanol (1 in 2) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, mix by shaking and use this solution as the standard solution. Perform the test with 10 μL of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and calculate the ratios, Q_T and Q_S, of the peak area of prednisolone succinate to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of prednisolone sodium succinate } (C_{25}H_{31}NaO_8) = \text{Amount (mg) of Prednisolone Succinate RS} \times \frac{Q_T}{Q_S} \times 5 \times 1.048$$

$$\text{Amount (mg) of prednisolone } (C_{21}H_{28}O_5) = \text{Amount (mg) of Prednisolone Succinate RS} \times \frac{Q_T}{Q_S} \times 5 \times 0.783$$

Internal standard solution—A solution of propyl *p*-hydroxybenzoate in diluted methanol (1 in 2) (1 in 25000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 ºC.

Mobile phase: Dissolve 0.32 g of tetra *n*-butylammonium bromide, 3.22 g of sodium dibasic phosphoric acid and 6.94 g of potassium monobasic phosphoric acid in 1000 mL of water. To 840 mL of this solution, add 1160 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of prednisolone succinate is about 15 minutes.

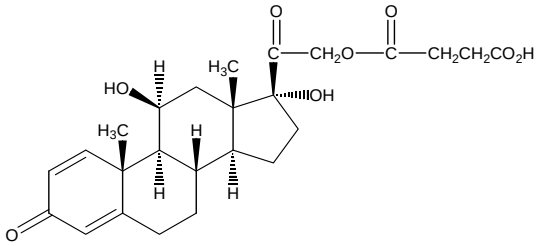
System suitanility

System performace: When the procedure is run with 10 μL of the standard solution under the above operating conditions, prednisolone succinate and the internal standard are eluted in this order with the resolution between their peaks being not less than 6.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of prednisolone Succinate to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Prednisolone Succinate



$C_{25}H_{32}O_8$: 460.52

4-(2-((8*S*,9*S*,10*R*,11*S*,13*S*,14*S*,17*R*)-11,17-dihydroxy-10,13-dimethyl-3-oxo-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[a]phenanthren-17-yl)-2-oxoethoxy)-4-oxobutanoic acid [*2920-86-7*]

Prednisolone Succinate, when dried, contains not less than 97.0 % and not more than 103.0 % of prednisolone succinate ($C_{25}H_{32}O_8$).

Description Prednisolone Succinate is a white, fine, crystalline powder and is odorless.
Prednisolone Succinate is freely soluble in methanol, soluble in ethanol (95) and very slightly soluble in water or in ether.

Melting point—About 205 ºC (with decomposition).

Identification (1) Take 2 mg of Prednisolone Succinate add 2 mL of sulfuric acid and allow to stand for 2 to 3 minutes: a deep red color, without fluorescence, develops. To this solution add 10 mL of water cautiously: the color disappears and a gray, flocculent precipitate is formed.

(2) Determine the infrared spectra of Prednisolone Succinate and Prednisolone Succinate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +114 ~ +120º (after drying, 67 mg, methanol, 10 mL, 100 mm).

Purity ***Related substances***—Dissolve 0.10 g of Prednisolone Succinate in methanol to make exactly 10 mL and use this solution as the test solution. Separately, dissolve 30 mg of prednisolone in methanol to make exactly 10 mL. Pipet 1.0 mL of the solution, add methanol to make exactly 10 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and ethanol (95) (2 : 1) to a distance of about 10 cm and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, P_2O_5, 60 ºC, 6 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 10 mg each of Prednisolone Succinate and Prednisolone Succinate RS, previously dried and dissolve each in methanol to make exactly 100 mL. Pipet 5 mL each of these solutions, add methanol to make exactly 50 mL and use these solutions as the test solution and the standard solution, respectively. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 242 nm as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of prednisolone succinate } (C_{25}H_{32}O_8) = \text{Amount (mg) of Prednisolone Succinate RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Tight containers.

Prednisolone Tablets

Prednisolone Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of prednisolone ($C_{21}H_{28}O_5$: 360.45).

Method of Preparation Prepare as directed under Tablets, with Prednisolone.

Identification (1) Weigh a portion of powdered Prednisolone Tablets, equivalent to 50 mg of prednisolone according to the labeled amount, add 10 mL of chloroform, shake for 15 minutes, and filter. Evaporate the filtrate on a water-bath to dryness. Dry the residue at 105ºC for 1 hour, and proceed with the residue as directed in the Identification (1) under Prednisolone.

(2) Determine the infrared spectra of the residue in (1) and Prednisolone RS, previously dried, as directed in the potassium brimide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears, dissolve the residue and Prednisolone RS in ethyl acetate, evaporate to dryness, and repeat the test on the residues.

Dissolution Test Take 1 tablet of Prednisolone Tablets, perform the test as directed in Method 2 under the Dissolution Test at 100 revolutions per minute, using 900 mL of water as the dissoution solution. After 20 minutes from start of the test, take 20 mL or more of the dissolved solution, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the test solution. Weigh accurately about 10 mg of Prednisolone RS, previously dried at 105 ºC for 3 hours, and dissolve in ethanol (95) to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at the wavelength of maximum absorption at about 242 nm as directed under Ultraviolet-visible Spectrophotometry.
The dissolution rate of Prednisolone Tablets in 20 minutes is not less than 70 %.

$$\text{Dissolution rate (\%) with respect to the labeled amount of prednisolone } (C_{21}H_{28}O_5) = W_S \times \frac{A_T}{A_S} \times \frac{45}{C}$$

W_S: Amount (mg) of Prednisolone RS.

C: Labeled amount (mg) of prednisolone ($C_{21}H_{28}O_5$) in 1 tablet.

Uniformity of Dosage Units It meets the requirement when the content uniformity test is performed according to the following procedure.

Take 1 tablet of Prednisolone Tablets, and shake with 10 mL of water until the tablet is disintegrated. Add 50 mL of methanol, shake for 30 minutes, add methanol to make exactly 100 mL, and centrifuge this solution. Pipet *x* mL of the clear supernatant liquid, add methanol to make exactly *V* mL to provide a solution that contains about 10 g of prednisolone ($C_{21}H_{28}O_5$) per mL, and use this solution as the test solution. Separately, weigh accurately about 10 mg of Prednisolone RS, previously dried at 105 ºC for 3 hours, dissolve in 10 mL of water and 50 mL of methanol, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 242 nm as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of prednisolone } (C_{21}H_{28}O_5) = \text{Amount (mg) of Prednisolone RS} \times \frac{A_T}{A_S} \times \frac{V}{10} \times \frac{1}{x}$$

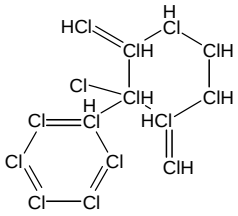
Assay Weigh accurately and powder not less than 20 Prednisolone Tablets using an agate mortar. Weigh accurately a portion of the powder, equivalent to about 5 mg of prednisolone ($C_{21}H_{28}O_5$), add 1 mL of water, and shake gently. Add 5.0 mL of the internal standard solution and 15 mL of methanol, and shake vigorously for 20 minutes. To 1.0 mL of this solution add the mobile phase to make exactlty 10 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrte, and use the subsequent filtrate as the test solution. Separately, weigh accurately about 25 mg of Prednisolone RS, previously dried at 105 ºC for 3 hours, dissolve in 50 mL of methanol, add 25.0 mL of the internal standard solution, and add methanol to make exactly 100 mL. To 1.0 mL of this solution add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Prednisolone.

$$\text{Amount (mg) of prednisolone } (C_{21}H_{28}O_5) = \text{Amount (mg) of Prednisolone RS} \times \frac{Q_T}{Q_S} \times \frac{1}{5}$$

Internal standard solution—A solution of methyl parahydoxybenzoate in methanol (1 in 2000)

Containers and Storage ***Containers***—Tight containers.

Primidone



$C_{12}H_{14}N_2O_2$: 218.25

5-Ethyl-5-phenyldihydropyrimidine-4,6(1*H*,5*H*)-dione [*125-33-7*]

Primidone, when dried, contains not less than 98.5 % and not more than 101.0 % of primidone ($C_{12}H_{14}N_2O_2$).

Description Primidone is a white, crystalline powder or granule, is odorless and has a slightly bitter taste.

Primidone is soluble in *N,N*-dimethylformamide, sparingly soluble in pyridine, slightly soluble in ethanol (95), very slightly soluble in water and practically insoluble in ether.

Identification (1) Heat 0.5 g of Primidone with 5 mL of diluted sulfuric acid (1 in 2): the odor of formaldehyde is perceptible.

(2) Mix 0.2 g of Primidone with 0.2 g of anhydrous sodium carbonate and heat: the gas evolved changes moistened red litmus paper to blue.

Melting Point 279 ~ 284 °C.

Purity (1) ***Clarity and color of solution***—Dissolve 0.10 g of Primidone in 10 mL of *N,N*-dimethylformamide: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 2.0 g of Primidone according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***2-Ethyl-2-phenylmalonediamide***—Dissolve 0.10 g of Primidone in 2 mL of pyridine, add 2.0 mL of the internal standard solution, then add 1 mL of bistrimethylsilyl acetamide, shake well and heat at 100 °C for 5 minutes. Cool, add pyridine to make 10 mL and use this solution as the test solution. Separately, dissolve 50 mg of 2-ethyl-2-phenylmalonediamide in pyridine to make exactly 100 mL. Pipet 2.0 mL of this solution, add 2.0 mL of the internal standard solution, proceed in the same manner as the test solution and use this solution as the standard solution. Perform the test with 2 μL of the test solution and the standard solution as directed under Gas Chromatography according to the following operating conditions and calculate the ratios, Q_T and Q_S, of the peak area of 2-ethyl-2-phenylmalonediamide to that of the internal standard for the test solution and the standard solution, respectively: Q_T is not more than Q_S.

Internal standard solution—A solution of stearyl alcohol in pyridine (1 in 2000).

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A column, about 3 mm in internal diameter and about 1.5 m in length, packed with siliceous earth for gas chromatography (125 μm to 150 μm in particle diameter), coated with 50 % phenyl-methyl silicone polymer at the ration of 3 %.

Column temperature: A constant temperature of about 195 °C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of stearyl alcohol is 8 to 9 minutes.

System suitability

System performance: When the procedure is run with 2 L of the standard solution under the above operating conditions, 2-ethyl-2-phenyl-malonediamide and the internal standard are eluted in this order with the resolution between their peaks being not less than 3.0.

System repeatability: When the test is repeated 5 times with 2 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of 2-ethyl-2-phenylmalonediamide to that of the internal standard is not more than 1.5 %.

(4) ***Related substances***—Weigh accurately about 100 mg of Primidone, dissolve in methanol to make exactly 100 mL, and use this solution as the test solution. Separately, weigh accurately about 100 mg of Primidone RS, dissolve in methanol to make exactly 100 mL, and use this solution as the standard solution (1). To 0.1 mL of the standard solution (1) add methanol to make exactly 100 mL, and use this solution as the standard solution (2). Perform the test with 10 μL each of the test solution and standard solution (2) as directed under Liquid Chromatography according to the following conditions. Determine each peak area of each solution by the automatic integration method, and calculate the amount of related substances in the test solution: primidone related substance I {2-ethyl-2-phenylmalonamide}, phenobarbital, primidone related substance II {2-phenylbutyramide}, 2-cyano-2-phenylbutylamide, and 2-phenylbutyric acid, having the relative retention times of about 0.5, about 1.4, about 1.6, about 1.8, and about 2.0 with respect to primidone, respectively, are not more than 0.1 %, respectively, and phenylpropylpimidone, having the relative retention time of about 2.8, is not more than 0.3 %. Any other related substance is not more than 0.1 %, and the total amount of related substances is not more than 0.5 %. Exclude any related substance not more than 0.05 %. Use the peak areas of primidone related substance I, primidone related substance II, 2-cyano-phenylbutylamide, and 2-phenylbutyric acid after dividing by their relative response factors, 0.67, 0.67, 0.71, and 0.77, respectively.

$$\text{Amount (\%) of each related substance} = 100 \times \frac{A_i}{A_S} \times \frac{C_S}{C_T}$$

A_i: Peak area of each related substance in the test solution

A_S: Peak area of primidone in the standard solution

C_S: Concentration (μg/mL) of primidone in the standard solution

C_T: Concentration (μg/mL) of primidone in the test solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 215 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: Dissolve 1.36 g of potassium dihydrogen phosphate in 1000 mL of water.

Mobile phase B: Methanol

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-1.0	75	25
1.0-6.0	75→40	25→60
6.0-8.0	40	60
8.0-8.5	40→75	60→25
8.5-10.0	75	25

Flow rate: 3.2 mL/minute

System suitability

System performance: When the procedure is run with 10 μL of the standard solution (2) under the above operating conditions, the symmetry factor of the peak of primidone is not more than 2. Dissolve 0.1 mg of Phenobarbital RS and 0.1 mg of Primidone Related Substance II RS in 10 mL of the standard solution (1), and use this solution as the system suitability solution. When the procedure is run with 10 μL of the system suitability solution under the above operating conditions, phenobarbital and primidone related substance II are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution (2) under the above operating conditions, the relative standard deviation of the peak area of primidone is not more than 5.0 %.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 20 mg each of Primidone and Primidone RS, previously dried, dissolve each in 20 mL of ethanol (95) by warming and after cooling, add ethanol (95) to make exactly 25 mL and use these solutions as the test solution and the standard solution, respectively. Determine the absorbance, A_1, of the test solution and the standard solution at the wavelength of a maximum absorption at about 257 nm and the absorbances, A_2 and A_3, at the wavelength of minimum absorption at about 254 nm and at about 261 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry, using ethanol (95) as the blank.

$$\text{Amount (mg) of primidone } (C_{12}H_{14}N_2O_2) = \text{Amount (mg) of Primidone RS} \times \frac{(2A_1 - A_2 - A_3)_T}{(2A_1 - A_2 - A_3)_S}$$

where $(2A_1-A_2-A_3)_T$ is the value from the test solution and $(2A_1-A_2-A_3)_S$ is from the standard solution.

Containers and Storage ***Containers***—Tight containers.

Primidone Tablets

Primidone Tablets contain not less than 95.0 % and not more than 105.0 % of the labeled amount of primidone ($C_{12}H_{14}N_2O_2$: 218.25).

Method of Preparation Prepare as directed under Tablets, with Primidone.

Identification The retention time of the principal peak in the chromatogram of the test solution corresponds to that of the standard solution, as obtained in the Assay.

Purity ***Related substances***—Weigh accurately and powder not less than 20 Primidone Tablets. Weigh accurately a portion of the powder, equivalent to about 250 mg of primidone ($C_{12}H_{14}N_2O_2$), and dissolve in 90 mL of methanol. Sonicate this solution for 15 minutes, then shake for about 20 minutes until all solids are dispersed. Cool this solution at an ordinary temperature, and add water to make exactly 250 mL. Filter through a filter with a pore size not exceeding 0.45 μm, discard the first 5 mL of the filtrate, and use the subsequent filtrate as the test solution. Separately, weigh accurately about 5 mg of Primidone RS, dissolve in methanol to make exactly 100 mL, and use this solution as the standard solution (1). To 2 mL of the standard solution (1) add the diluent to make exactly 50 mL, and use this solution as the standard solution (2). Perform the test with 20 μL each of the test solution and standard solution (2) as directed under Liquid Chromatography according to the following conditions. Determine each peak area of each solution by the automatic integration method, and calculate the amount of related substances in the test solution: primidone related substance I {2-ethyl-2-phenylmalonamide}, phenobarbital, primidone related substance II, and 2-phenylbutyric acid, having the relative retention times of about 0.5, about 1.6, about 1.9, and about 4.1 with respect to primidone, respectively, are not less than 0.1 %, respectively. Any other related substance is not more than 0.1 %, and the total amount of related substances is not more than 0.3 %. Exclude any related substance not more than 0.025 %. Use the peak areas of primidone related substance I, primidone related substance II, and 2-phenylbutyric acid after dividing by their relative response factors, 0.76, 0.92, and 0.91, respectively.

$$\text{Amount (\%) of each related substance} = 100 \times \frac{A_i}{A_S} \times \frac{C_S}{C_T}$$

A_i: Peak area of each related substance in the test solution

A_S: Peak area of primidone in the standard solution

C_S: Concentration (μg/mL) of primidone in the standard solution

C_T: Concentration (μg/mL) of primidone in the test solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 35 °C

Mobile phase: A mixture of a solution of potassium dihydrogen phosphate (6.8 in 1000), tetrahydrofuran, and methanol (65 : 0.5 : 35)

Flow rate: 1.3 mL/minute

System suitability

System performance: Dissolve 20 mg of Primidone Related Substance I RS in 100 mL of methanol. To 10 mL of this solution add the diluent to make excatly 100 mL, and use this solution as primidone related substance I standard solution. Dissolve 1 mL of primidone related substance I standard solution and 0.8 mL of the standard solution (2) in the diluent to make exactly 20 mL, and use this solution as the system suitability solution. When the procedure is run with 20 μL of this solution under the above operating conditions, the resolution between the peaks of primidone related substance I and primidone is not less than 4.0.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution (2) under the above operating conditions, the relative standard deviation of the peak area of primidone is not more than 5.0 %.

Diluent—A mixture of methanol and water (35 : 65)

Dissolution Test Perform the test with 1 tablet of Primidone Tablets at 50 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of water as the dissolution solution. Take the dissolved solution after 60 minutes from start of the test, filter, dilute with the dissolution solution, if necessary and use this solution as the test solution. Separately, weigh accurately a portion of Primidone RS, previously dried at 105 °C for 2 hours, add the water to make the same concentration with the dissolution solution and use this solution as the standard solution. Determine the absorbances of the test solution and the standard solution at 257 nm as directed under Ultraviolet-visible Spectrophotometry using the water as the blank. The dissolution rate of Primidone Tablets in 60 minutes is not less than 75 %.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Primidone Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of primidone ($C_{12}H_{14}N_2O_2$), add 35 mL of ethanol (95) and boil for 1 hour. Cool to room temperature, add 5.0 mL of the internal standard solution, add ethanol (95) to make exactly 50 mL, filter and use this solution as the test solution. Separately, weigh accurately about 50 mg of Primidone RS, previously dried at 105 °C for 2 hours, add 35 mL of ethanol (95) and boil for 1 hour. Cool to room temperature, add 5.0 mL of the internal standard solution, add ethanol (95) to make exactly 50 mL and use this solution as the standard solution. Perform the test with 3 μL each of the test solution and the standard solution as directed under Gas Chromatography according to the following operating conditions and calculate the ratios, Q_T and Q_S, of the peak area of Primidone to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of primidone } (C_{12}H_{14}N_2O_2) = \text{Amount (mg) of Primidone RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of androsterone in ethanol (95) (1 in 100).

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A glass column, about 4.0 mm in internal diameter and about 120 cm in length, packed with siliceous earth, coated with 50 % phenyl-50 % methylpolysiloxane at the ratio of 10 %.

Column temperature: About 260 °C.

Injector and detector temperature: About 310 °C.

Carrier gas: Helium.

Flow rate: 40 mL/minute.

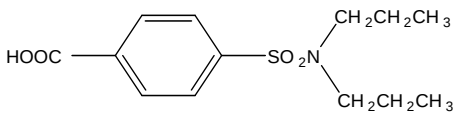
System suitability

System performance: When the procedure is run with 3 μL of the standard solution according to the above operating conditions, the resolution between the peaks of primidone and the internal standard is not less than 1.5.

System repeatability: When the test is repeated 6 times with 3 μL each of the standard solution according to the above operating conditions, the relative standard deviation of the ratios of the peak area of primidone to that of the internal standard is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Probenecid



$C_{13}H_{19}NO_4S$: 285.36

4-(Dipropylsulfamoyl)benzoic acid [*57-66-9*]

Probenecid, when dried, contains not less than 98.0 % and not more than 101.0 % of probenecid ($C_{13}H_{19}NO_4S$).

Description Probenecid appears as white crystals or crystalline powder, is odorless and has a slightly bitter taste, followed by unpleasant bitter taste.
Probenecid is sparingly soluble in ethanol (95), slightly soluble in ether and practically insoluble in water.
Probenecid dissolves in sodium hydroxide TS or in ammonia TS.

Melting point—198 ~ 200 ºC

Identification (1) Heat Probenecid strongly: the odor of sulfur dioxide is perceptible.

(2) Determine the absorption spectra of a solution of Probenecid and Probenecid RS in ethanol (95) (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) ***Acid***—Take 2.0 g of Probenecid, add 100 mL of water, heat in a water-bath with occasional shaking for 30 minutes, cool and filter. To the filtrate, add 1 drop of phenolphthalein TS and 0.50 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(2) ***Chloride***—Take 1.0 g of Probenecid, add 100 mL of water and 1 mL of nitric acid and heat in a wa-

ter-bath with occasional shaking for 30 minutes. After cooling, add, if necessary, water to make 100 mL and filter. Perform the test with 50 mL of the filtrate as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021 %).

(3) ***Sulfate***—Take 1.0 g of Probenecid, add 100 mL of water and 1 mL of hydrochloric acid and heat in a water-bath with occasional shaking for 30 minutes. After cooling, add, if necessary, water to make 100 mL and filter. Perform the test using 50 mL of the filtrate as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038 %).

(4) ***Heavy metals***—Proceed with 2.0 g of Probenecid according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(5) ***Arsenic***—Prepare the test solution with 1.0 g of Probenecid according to Method 3 and perform the test (not more than 2 ppm).

(6) ***Selenium***—Weigh accurately 0.1 g of Probenecid, add 0.1 g of magnesium oxide, mix, transfer to a combustion flask, and proceed as directed under Oxygen Flask Combustion Method, using 25 mL of diluted nitric acid (1 in 30) as the absorbing liquid. Use a 1000 mL combustion flask. After combustion, wash the stopper and the inside of the flask with 10 mL of water. Transfer the liquid inside the flask to a 150 mL beaker, using about 20 mL of water. Heat gently to boil, boil for 10 minutes, cool to room temperature, and use this solution as the test solution. Separately, pipet 3.0 mL of selenium standard stock solution, add 25 mL of diluted nitric acid (1 in 30) and 25 mL of water, and use this solution as the standard solution. Adjust the pH of the test solution and standard solution to 2.0 ± 0.2 with diluted ammonia solution (28) (1 in 2), add water to make exactly 60 mL, transfer to a separatory funnel using 10 mL of water, and wash the separatory funnel with 10 mL of water. Add 0.2 g of hydroxylamine hydrochloride, stir to dissolve, add immediately 5.0 mL of 2,3-diaminonaphthalene TS, stopper, stir to mix, and allow to stand at room temperature for 100 minutes. Add 5.0 mL of cyclohexane, shake vigorously for 2 minutes, allow the layers to separate, discard the water layer, centrifuge the cyclohexane extract to remove water, and take the cyclohexane layer. Determine the absorbances at 380 nm of these solutions as directed under Ultraviolet-visible Spectrophotometry, using a solution prepared by adding 25 mL of water to 25 mL of diluted nitric acid (1 in 30) and proceeding in the same manner, as the blank: the absorbance obtained from the test solution is not more than that from the standard solution (not more than 30 ppm).

(7) ***Related substances***—Weigh accurately 50 mg of Probenecid, add the mobile phase to make 100 mL, and use this solution as the test solution. Perform the test with 20 μL of the test solution as directed under Liquid Chromatography according to the following conditions, determine the area of each peak other than the solvent by the automatic integration method, and calculate the amount of each related substance by the area percentage method: each related substance is not more than 0.5 %, and the total amount of related substances is not more than 2.0 %.

$$\text{Amount (\%) of each related substance} = 100 \times \frac{A_i}{A_S}$$

A_i: Peak area of each related substance obtained from the test solution

A_S: Total area of all peaks obtained from the test solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 3.9 mm in internal diameter and about 30 cm in length, packed with phenylsilyl silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of sodium phosphate solution and a solution of acetic acid (100) in acetonitrile (1 in 100) (1 : 1)

Flow rate: 1 mL/minute

System suitability

System performance: Weigh accurately 50 mg of Probenecid RS, add the mobile phase to make 100 mL, and use this solution as the system suitability solution. When the procedure is run with 20 μL of this solution under the above operating conditions, the number of theoretical plates and symmetry factor are not less than 3900 and not more than 2.3, respectively.

System repeatability: When the test is repeated 6 times with 20 μL each of the system suitability solution under the above operating conditions, the relative standard deviation is not more than 1.5 %.

Sodium phosphate solution—Prepare a 0.05 mol/L solution of sodium dihydrogen phosphate using a solution of acetic acid (100) (1 in 100) as the solvent, then adjust the pH to 3.0 with phosphoric acid.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.5 g of Probenecid, previously dried and dissolve in 50 mL of neutralized ethanol. Titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 28.536 mg of $C_{13}H_{19}NO_4S$

Containers and Storage ***Containers***—Well-closed containers.

Probenecid Tablets

Probenecid Tablets contain not less than 95.0 % and not more than 105.0 % of the labeled amount of probenecid ($C_{13}H_{19}NO_4S$: 285.36).

Method of Preparation Prepare as directed under Tablets, with Probenecid.

Identification (1) Weigh a portion of powdered Probenecid Tablets, equivalent to 0.5 g of probenecid according to the labeled amount, add 50 mL of ethanol (95) and 1 mL of 1 mol/L hydrochloric acid TS, shake and filter. Evaporate the filtrate in a water-bath to about 20 mL. After cooling, filter and collect the produced crystals, recrystallize from 50 mL of dilute ethanol and dry at 105 ºC for 4 hours: it melts between 198 ºC and 200 ºC. With the crystals so obtained, proceed as directed in the Identification (1) under Probenecid.

(2) Determine the absorption spectrum of a solution of the dried crystals obtained in Identification (1) in ethanol (95) (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 224 nm and 226 nm and between 247nm and 249 nm and a minimum between 234 nm and 236 nm.

Dissolution Test Perform the test with 1 tablet of Probenecid Tablets at 50 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of the 2nd fluid for dissolution test as the dissolution solution. Take 30 mL or more of the dissolved solution after 30 minutes from start of the test and filter through a membrane filter with a pore size of not more than 0.8 μm. Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add diluted phosphate buffer solution, pH 6.8 (1 in 2), to make exactly V' mL so that each mL contains about 14 μg of probenecid ($C_{13}H_{19}NO_4S$) according to the labeled amount and use this solution as the test solution. Separately, weigh accurately about 70 mg of Probenecid RS, previously dried at 105 ºC for 4 hours and add the 2nd fluid for dissolution test to make exactly 100 mL. Pipet 1 mL of this solution, add the 2nd fluid for dissolution test to make exactly 50 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 244 nm as directed under Ultraviolet-visible Spectrophotometry.
The dissolution rate of Probenecid Tablets in 30 minutes is not less than 80 %.

$$\text{Dissolution rate (\%) with respect to the labeled amount of probenecid } (C_{13}H_{19}NO_4S) = W_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 18$$

W_S: Amount (mg) of Probenecid RS,
C: Labeled amount (mg) of probenecid ($C_{13}H_{19}NO_4S$) in 1 tablet.

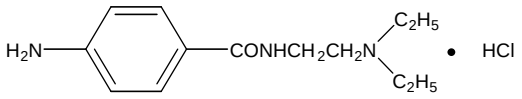
Uniformity of Dosage Units It meets the repuirement.

Assay Weigh accurately and powder not less than 20 Probenecid Tablets. Weigh accurately a portion of the powder, equivalent to about 0.25 g of probenecid ($C_{13}H_{19}NO_4S$), dissolve in 30 mL of water and 2 mL of 1 mol/L hydrochloric acid TS, and add 30 mL of ethanol (99.5). Add ethanol (99.5) to make exactly 100 mL, centrifuge, pipet 3 mL of the supernatant liquid, and add 1 mL of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) to make exactly 50 mL. Pipet 5 mL of this solution, add ethanol (99.5) to make exactly 50 mL, and use this solution as the test solution. Separately, weigh accurately about 0.125 g of Probenecid RS (previously dried at 105 °C for 4 hours), dissolve in 1 mL of 1 mol/L hydrochloric acid TS and 15 mL of water, and add ethanol (99.5) to make exactly 50 mL. Pipet 3 mL of this solution, add 2 mL of 1 mol/L hydrochloric acid TS, and add ethanol (99.5) to make exactly 50 mL. Pipet 5 mL of this solution, add ethanol (99.5) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 248 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry, using as the blank a solution prepared by adding ethanol (99.5) to 1 mL of 0.1 mol/L hydrochloric acid TS to make exactly 50 mL.

$$\text{Amount (mg) of probenecid } (C_{13}H_{19}NO_4S) = \text{Amount (mg) of Probenecid RS} \times \frac{A_T}{A_S} \times 2$$

Containers and Storage ***Containers***—Well-closed containers.

Procainamide Hydrochloride



$C_{13}H_{21}N_3O \cdot HCl$: 271.79

4-Amino-*N*-(2-diethylaminoethyl)benzamide hydrochloride [*614-39-1*]

Procainamide Hydrochloride, when dried, contains not less than 98.0 % and not more than 101.0 % of procainamide hydrochloride ($C_{13}H_{21}N_3O \cdot HCl$).

Description Procainamide Hydrochloride appears as white to pale yellow crystalline powder.
Procainamide Hydrochloride is very soluble in water, and soluble in ethanol (99.5).
Procainamide Hydrochloride is hygroscopic.

Identification (1) Determine the infrared spectra of Procainamide Hydrochloride and Procainamide Hydrochloride RS as directed in the potassium chloride disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Procainamide Hydrochloride (1 in 20) responds to the Qualitative Tests for chloride.

Melting Point 165 ~ 169 °C.

pH Dissolve 1.0 g of Procainamide Hydrochloride in 10 mL of water: the pH of this solution is between 5.0 and 6.5.

Purity (1) ***Clarity and color of solution*** —Dissolve 1.0 g of Procainamide Hydrochloride in 10 mL of water: the solution is clear.

(2) ***Heavy metals***—Proceed with 2.0 g of Procainamide Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Procainamide Hydrochloride according to Method 1 and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 50 mg of Procainamide Hydrochloride in 100 mL of the mobile phase, and use this solution as the test solution. Pipet 1 mL of this solution, and add the mobile phase to make exactly 50 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine peak areas by the area percentage method: the total area of the peaks other than procainamide hydrochloride from the test solution is not larger than the peak area of procainamide hydrochloride from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 270 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: A mixture of 0.02 mol/L phosphate buffer solution (pH 3.0) and methanol (9 : 1)

Flow rate: Adjust the flow rate so that the retention time of procainamide hydrochloride is 9 minutes.

System suitability

Test for required detectability: Pipet 10 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of procainamide obtained from 10 μL of this solution is equivalent to 40 to 60 % of that from the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of procainamide are not less than 10000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of procainamide is not more than 2.0 %.

Time span of measurement: About 2 times as long as the retention time of procainamide.

Loss on Drying Not more than 0.3 % (2 g, 105 °C, 4 hours).

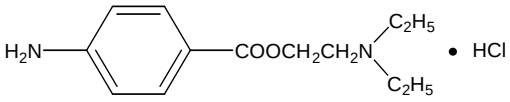
Residue on Ignition Not more than 0.1 % (2 g).

Assay Weigh accurately about 0.5 g of Procainamide Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 27.179 mg of $C_{13}H_{21}N_3O \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Procaine Hydrochloride



$C_{13}H_{20}N_2O_2 \cdot HCl$: 272.77

2-(Diethylamino)ethyl4-aminobenzoate hydrochloride [*51-05-8*]

Procaine Hydrochloride, when dried, contains not less than 99.0 % and not more than 101.0 % of procaine hydrochloride ($C_{13}H_{20}N_2O_2 \cdot HCl$).

Description Procaine Hydrochloride appears as white crystals or crystalline powder.
Procaine Hydrochloride is very soluble in water, soluble in ethanol (95) and practically insoluble in ether.

Identification (1) Determine the absorption spectra of a solution of Procaine Hydrochloride and Procaine Hydrochloride RS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Procaine Hydrochloride and Procaine Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Procaine Hydrochloride (1 in 10) responds to the Qualitative Tests for chloride.

Melting Point 155 ~ 158 °C.

pH Dissolve 1.0 g of Procaine Hydrochloride in 20 mL of water: the pH of this solution is between 5.0 and 6.0.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Procaine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Procaine Hydrochloride according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Take 1.0 g of Procaine Hydrochloride, add 5 mL of ethanol (95), dissolve by mixing well, add water to make exactly 10 mL and use this solution as the test solution. Separately, dissolve 10 mg of 4-aminobenzoic acid in ethanol (95) to make exactly 20 mL, then pipet 1 mL of this solution, add 4 mL of ethanol (95) and water to make exactly 10 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dibutyl ether, n-hexane and acetic acid (100) (20 : 4 : 1) to a distance of about 10 cm and air-dry the plate. After drying the plate at 105 °C for 10 minutes, examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more dense than the spot from the standard solution. The principal spot from the test solution stays at the origin.

Loss on Drying Not more than 0.5 % (1 g, silica gel, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.4 g of Procaine Hydrochloride, previously dried, dissolve in 5 mL of hydrochloric acid and 60 mL of water, add 10 mL of a solution of potassium bromide (3 in 10), cool to below 15 °C and titrate with 0.1 mol/L sodium nitrite VS (potentiometric titration or amperometric titration, Endpoint Detection Method in Titrimetry).

Each mL of 0.1 mol/L sodium nitrite VS
= 27.277 mg of $C_{13}H_{20}N_2O_2 \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Procaine Hydrochloride Injection

Procaine Hydrochloride Injection is an aqueous solution for injection. Procaine Hydrochloride Injection contains not less than 95.0 % and not more than 105.0 % of the labeled amount of procaine hydrochloride ($C_{13}H_{20}N_2O_2 \cdot HCl$: 272.77).

Method of Preparation Prepare as directed under Injections, with Procaine Hydrochloride.

Description Procaine Hydrochloride Injection is a clear, colorless liquid.

Identification (1) Take a volume of Procaine Hydrochloride Injection, equivalent to 10 mg of Procaine Hydrochloride according to the labeled amount and add water to make 1000 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 219 nm and 223 nm and between 289 nm and 293 nm.

(2) Procaine Hydrochloride Injection responds to the Qualitative Tests (2) for chloride.

pH 3.3 ~ 6.0.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.6 EU/mg of procaine hydrochloride. Less than 0.02 EU/mg of procaine hydrochloride for preparations intended for intraspinal administration.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay Take an exactly measured volume of Procaine Hydrochloride Injection, equivalent to about 20 mg of procaine hydrochloride ($C_{13}H_{20}N_2O_2 \cdot HCl$), add the mobile phase to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and the mobile phase to make exactly 20 mL and use this solution as the test solution. Separately, weigh accurately about 20 mg of Procaine Hydrochloride RS, previously dried in a desiccator (silica gel) for 4 hours and dissolve in the mobile phase to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and the mobile

phase to make exactly 20 mL and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and calculate the ratios, Q_T and Q_S, of the peak area of procaine to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of procaine hydrochloride } (C_{13}H_{20}N_2O_2\cdot HCl) = \text{Amount (mg) of Procaine Hydrochloride RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of caffeine in the mobile phase (1 in 1000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate to 3.0 with phosphoric acid and add an amount of sodium-1-heptanesulfonate to make a solution of 0.1 %. Add 200 mL of methanol to 800 mL of this solution.

Flow rate: Adjust the flow rate so that the retention time of procaine hydrochloride is about 10 minutes.

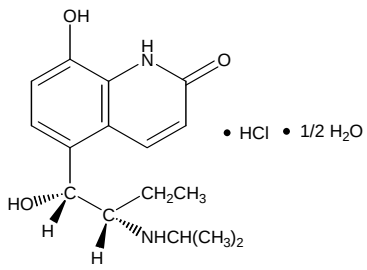
System suitability

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, procaine and the internal standard are eluted in this order with the resolution between their peaks being not less than 8.0.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pracaine to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Procaterol Hydrochloride Hydrate



$C_{16}H_{22}N_2O_3\cdot HCl\cdot {}^1/_2H_2O$: 335.83

(1*RS*,2*SR*)-8-Hydroxy-5-[1-hydroxy-2-(isopropylamino)butyl]-quinolin-2(1*H*)-one hydrochloride hemihydrate [*62929-91-3,* anhydride]

Procaterol Hydrochloride Hydrate contains not less than 98.5 % and not more than 101.0 % of procaterol hydrochloride ($C_{16}H_{22}N_2O_3\cdot HCl$: 326.82), calculated on the anhydrous basis.

Description Procaterol Hydrochloride Hydrate appears as white pale yellowish white crystals or crystalline powder.

Procaterol Hydrochloride Hydrate is soluble in water, in formic acid or in methanol, slightly soluble in ethanol (95) and practically insoluble in ether.

pH—The pH of a solution of Procaterol Hydrochloride Hydrate (1 in 100) is between 4.0 and 5.0.

Procaterol Hydrochloride Hydrate is gradually affected by light.

The solution of Procaterol Hydrochloride Hydrate (1 in 20) shows no optical rotation.

Melting point—About 195 °C (with decomposition).

Identification (1) Determine the absorption spectra of solutions of Procaterol Hydrochloride Hydrate and Procaterol Hydrochloride Hydrate RS (7 in 1000000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Procaterol Hydrochloride Hydrate and Procaterol Hydrochloride Hydrate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Procaterol Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests for chloride.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Procaterol Hydrochloride Hydrate in 30 mL of water: the solution is clear and has no more color than the following control solution.

Control solution—Take 3.0 mL of iron (III) chloride hexahydrate stock CS, add water to make 50 mL.

(2) ***Heavy metals***—Proceed with 2.0 g of Procaterol Hydrochloride Hydrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Dissolve 0.10 g of Procaterol Hydrochloride Hydrate in 100 mL of diluted methanol (1 in 2) and use this solution as the test solution. Pipet 1.0 mL of the test solution, add diluted methanol (1 in 2) to make exactly 100 mL and use this solution as the standard solution. Perform the test with 2 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine each peak area of the test solution and the standard solution by the automatic integration method: the total area of the peaks other than procaterol from the test solution is not larger than the peak area of procaterol from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 25 cm in length packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: Dissolve 0.87 g of sodium-1-heptanesulfonate in 1000 mL of water. To 760 mL of this solution, add 230 mL of methanol and 10 mL of acetic acid (100).

Flow rate: Adjust the flow rate so that the retention time of procaterol is about 15 minutes.

System suitability

Detection sensitivity: Adjust the detection sensitivity so that the peak height of procaterol obtained from 2 μL of the standard solution is not less than 10 mm.

System performance: Dissolve 20 mg each of Procaterol Hydrochloride Hydrate and threoprocaterol hydrochloride in 100 mL of diluted methanol (1 in 2). To 15 mL of this solution, add diluted methanol (1 in 2) to make 100 mL. When the procedure is run with 2 μL of this solution according to the above operating conditions, procaterol and threoprocaterol are eluted in this order with the resolution between their peaks being not less than 3.0.

Time span of measurement: 2.5 times as long as the retention time of procaterol after the solvent peak.

Water 2.5 ~ 3.3 % (0.5 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

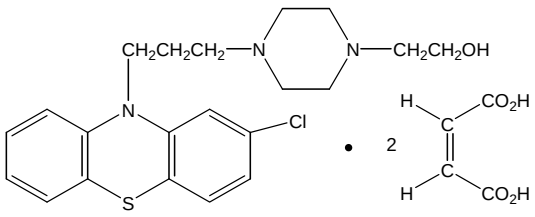
Assay Weigh accurately about 0.25 g of Procaterol Hydrochloride Hydrate, add 2 mL of formic acid, dissolve by warming and add exactly 15 mL of 0.1 mol/L perchloric acid VS. Add 1 mL of acetic anhydride, heat on a water-bath for 30 minutes, cool, add 60 mL of acetic anhydride and titrate the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.682 mg of $C_{16}H_{22}N_2O_3 \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Prochlorperazine Maleate



$C_{20}H_{24}ClN_3S \cdot 2C_4H_4O_4$: 606.09

2-Chloro-10-[3-(4-methyl-1-piperazinyl)propyl]-10*H*-phenothiazine (*Z*)-but-2-enedioate [*84-02-6*]

Prochlorperazine Maleate, when dried, contains not less than 98.0 % and not more than 101.0 % of prochlorperazine maleate ($C_{20}H_{24}ClN_3S \cdot 2C_4H_4O_4$).

Description Prochlorperazine Maleate is a white to pale yellow powder, is odorless and has a slightly bitter taste.

Prochlorperazine Maleate is slightly soluble in acetic acid (100), very slightly soluble in water or in ethanol (95) and practically insoluble in ether.

Prochlorperazine Maleate is gradually colored to a red-tint by light.

Melting point—195 ~ 203 °C (with decomposition).

Identification (1) Dissolve 5 mg of Prochlorperazine Maleate in 5 mL of sulfuric acid: a red color is observed, which darkens slowly on standing. Warm a half of the solution: the color changes to red-purple. To the remainder, add 1 drop of potassium dichromate TS: a green-brown color is observed, which changes to brown on standing.

(2) Dissolve 0.2 g of Prochlorperazine Maleate in 5 mL of a solution of sodium hydroxide (1 in 10) and extract with three 3 mL volumes of ether [reserve the aqueous layer and use for (4)]. Evoporate the combined ether extracts on a water-bath to dryness, dissolve the

residue in 10 mL of methanol by warming and pour into 30 mL of a solution of 2,4,6-trinitrophenol in methanol (1 in 75), previously warmed to 50 °C. Allow to stand for 1 hour, filter and collect the crystals, wash with a small amount of methanol and dry at 105 °C for 1 hour: the crystals melt between 252 °C and 258 °C (with decomposition).

(3) Boil 0.5 g of Prochlorperazine Maleate with 10 mL of hydrobromic acid under a reflux condenser for 10 minutes. After cooling, add 100 mL of water and filter through glass filter (G4). Wash the residue with three 10 mL volumes of water and dry at 105 °C for 1 hour: it melts between 195 °C and 198 °C (with decomposition).

(4) To the aqueous layer reserved in (2), add boiling chips and heat on a water-bath for 10 minutes. Cool, add 2 mL of bromine TS, heat on a water-bath for 10 minutes and heat the solution to boil. After cooling, add 2 drops of this solution to 3 mL of a solution of resorcinol in sulfuric acid (1 in 300) and heat on a water-bath for 15 minutes: a red-purple color is observed.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Prochlorperazine Maleate according to Method 2 and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Weigh accurately 100 mg of Prochlorperazine Maleate, dissolve in 10 mL of a mixture of methanol and 1 mol/L sodium hydroxide (9 : 1), and use this solution as the test solution. Separately, weigh accurately 10 mg of Prochlorperazine Maleate RS, dissolve in 50 mL of a mixture of methanol and 1 mol/L sodium hydroxide (9 : 1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 20 μL each of the test solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia solution (28) (100 : 1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm and 365 nm): any spot other than the principal spot obtained from the test solution is not larger or more intense than the spot from the standard solution (not more than 2.0 %). Disregard any spot remaining at the origin line.

Loss on Drying Not more than 1.0 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

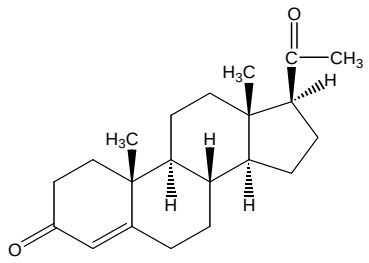
Assay Weigh accurately about 0.3 g of Prochlorperazine Maleate, previously dried and dissolve in 60 mL of acetic acid (100), while stirring and warming. Cool and titrate with 0.05 mol/L perchloric acid VS until the color of the solution changes from orange to green (indicator: 0.5 mL of 1-naphtholbenzeine TS). Perform a blank determination and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 15.152 mg of $C_{20}H_{24}ClN_3S \cdot 2C_4H_4O_4$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Progesterone



$C_{21}H_{30}O_2$: 314.46

(8*S*,9*S*,10*R*,13*S*,14*S*,17*S*)-17-Acetyl-10,13-dimethyl-1,2,6,7,8,9,11,12,14,15,16,17-dodecahydrocyclopenta[a]phenanthren-3-one [*57-83-0*]

Progesterone, when dried, contains not less than 97.0 % and not more than 103.0 % of progesterone ($C_{21}H_{30}O_2$).

Description Progesterone appears as white crystals or crystalline powder.
Progesterone is soluble in methanol or in ethanol (99.5), and practically insoluble in water.

Identification (1) Determine the absorption spectra of solutions of Progesterone and Progesterone RS in ethanol (99.5) (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Progesterone and Progesterone RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry,: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears, dissolve each in ethanol (95), evaporate to dryness and repeat the test on the residues.

Specific Optical Rotation $[\alpha]_D^{20}$: +184 ~ +194° (0.2 g after drying, ethanol (99.5), 10 mL, 100 mm).

Melting Point 128 ~ 133 ºC or 120 ~ 122 ºC

Purity ***Related substances***—Dissolve 80 mg of Progesterone in 2 mL of methanol and use this solution as the test solution. Pipet 1 mL of this solution, add methanol to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test

solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and diethylamine (19 : 1) to a distance of about 15 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (0.5 g, in vacuum, P_2O_5, 4 hours)

Residue on Ignition Not more than 0.1 % (0.5 g).

Assay Weigh accurately about 10 mg each of Progesterone and Progesterone RS, previously dried, and dissolve each in ethanol (99.5) to make exactly 100 mL. Pipet 5 mL each of these solutions, add ethanol (99.5) to make exactly 50 mL, and use these solutions as the test solution and standard solution. Determine the absorbances, A_T and A_S, at 241 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of progesterone } (C_{21}H_{30}O_2) = \text{Amount (mg) of Progesterone RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Progesterone Injection

Progesterone Injection is an oily solution for injection. Progesterone Injection contains not less than 90.0 % and not more than 110.0 % of the labeled amount of progesterone ($C_{21}H_{30}O_2$: 314.46).

Method of Preparation Prepare as directed under Injections, with Progesterone.

Description Progesterone Injection is a clear, colorless to pale yellow, oily liquid.

Identification To 1 mL of Progesterone Injection add 1 mL of diluted ethanol (9 in 10), shake well, take the ethanol layer, add 1 mL of petroleum benzene, shake, and use the ethanol layer as the test solution. Separately, dissolve about 5 mg of Progesterone RS in 1 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 2 μL each of the test solution and standard solution on a plate of silica gel for liquid chromatography. Develop the plate with a mixture of ether and diethylamine (19 : 1) to a distance of about 10 cm, and air-dry the plate. Spray evenly sulfuric acid on the plate, and heat at 105 °C for 10 minutes: the principal spot obtained from the test solution has the same R_f value as the principal spot from the standard solution.

Sterility Test It meets the requirement.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay Determine the specific gravity of Progesterone Injection. Weigh accurately an amount of Progesterone Injection, equivalent to 1 mL, add 2 mL of tetrahydrofuran, shake, and add ethanol (99.5) to make exactly V mL so that each mL contains 0.5 mg of progesterone ($C_{21}H_{30}O_2$). Pipet 2 mL of this solution, add 10 mL of the internal standard solution, add ethanol (99.5) to make exactly 20 mL, and use this solution as the test solution. Separately, weigh accurately about 10 mg of Progesterone RS, previously dried in a desiccator (in vacuum, P_2O_5, 4 hours), dissolve in 2 mL of tetrahydrofuran, and add ethanol (99.5) to make exactly 20 mL. Pipet 2 mL of this solution, add 10 mL of the internal standard solution and ethanol (99.5) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of progesterone to that of the internal standard in each solution.

$$\text{Amount (mg) of progesterone } (C_{21}H_{30}O_2) \text{ in each mL of Progesterone Injection} = \text{Amount (mg) of Progesterone RS} \times \frac{Q_T}{Q_S} \times \frac{V}{20}$$

Internal standard solution—A solution of testosterone propionate in ethanol (99.5) (1 in 4000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 241 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35 °C

Mobile phase: A mixture of acetonitrile and water (7 : 3)

Flow rate: Adjust the flow rate so that the retention time of progesterone is about 6 minutes.

System suitability

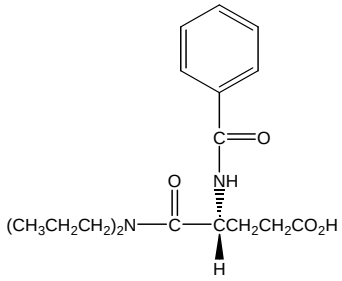
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, progesterone and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of progesterone to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Storage—Light-resistant.

Proglumide



and enantiomer

$C_{18}H_{26}N_2O_4$: 334.41

(4*RS*)-4-Benzamido-5-(dipropylamino)-5-oxopentanoic acid [*6620-60-6*]

Proglumide, when dried, contains not less than 98.5 % and not more than 101.0 % of proglumide ($C_{18}H_{26}N_2O_4$).

Description Proglumide appears as white crystals or crystalline powder.

Proglumide is freely soluble in methanol, soluble in ethanol (95), slightly soluble in ether, and practically insoluble in water.

A solution of Proglumide in methanol (1 in 10) shows no optical rotation.

Identification (1) Put 0.5 g of Proglumide in a round-bottomed tube, add 5 mL of hydrochloric acid, seal the tube and heat the tube carefully at 120 ºC for 3 hours. After cooling, open the tube, collect crystals separated out, wash the crystals with 50 mL of water and dry at 100 ºC for 1 hour: the melting point of the crystals is between 121 ºC and 124 ºC.

(2) Determine the infrared spectra of Proglumide and Proglumide RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 148 ~ 150 ºC.

Absorbance $E_{1cm}^{1\%}$ (225 nm): 384 ~ 414 (after drying, 4 mg, methanol, 250 mL)

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Proglumide according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Arsenic***—Take 1.0 g of Proglumide, add 10 mL of a solution of magnesium nitrate in ethanol (95) (1 in 10) and 1.5 mL of hydrogen peroxide solution, burn the ethanol and prepare the test solution according to Method 3 and perform the test (not more than 20 ppm).

(3) ***Related substances***—Dissolve 0.10 g of Proglumide in 5 mL of methanol and use this solution as the test solution. Pipet 1 mL of the test solution, add methanol to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, ethyl acetate, acetic acid (100) and methanol (50 : 18 : 5 : 4) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.1 % (1 g, in vaccum, P_2O_5, 60 ºC, 3 hours).

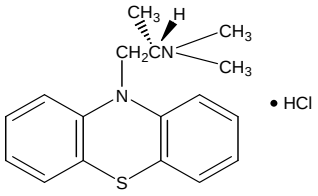
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.16 g of Proglumide, previously dried, dissolve in 40 mL of methanol, add 10 mL of water and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 33.441 mg of $C_{18}H_{26}N_2O_4$

Containers and Storage ***Containers***—Well-closed containers.

Promethazine Hydrochloride



and enantiomer

$C_{17}H_{20}N_2S \cdot HCl$: 320.88

(*RS*)-*N*,*N*-dimethyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine hydrochloride [*58-33-3*]

Promethazine Hydrochloride, when dried, contains not less than 98.0 % and not more than 101.0 % of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$).

Description Promethazine Hydrochloride is a white to pale yellow powder.
Promethazine Hydrochloride is very soluble in water, freely soluble in ethanol (95) or in acetic acid (100), sparingly soluble in acetic anhydride and practically insoluble in ether.
Promethazine Hydrochloride is gradually colored by light.
A solution of Promethazine Hydrochloride (1 in 25) shows no optical rotation.

Melting point—About 223 °C (with decomposition).

Identification (1) Determine the absorption spectra of solutions of Promethazine Hydrochloride and Promethazine Hydrochloride RS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Promethazine Hydrochloride and Promethazine Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Dissolve 0.5 g of Promethazine Hydrochloride in 5 mL of water, add 2 mL of ammonia TS and filter. To 5 mL of the filtrate, add dilute nitric acid to make acidic: the solution responds to the Qualitative Tests (2) for chloride.

pH Dissolve 1.0 g of Promethazine Hydrochloride in 10 mL of water: the pH of this solution is between 4.0 and 5.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Promethazine Hydrochloride in 10 mL of water, protecting form direct sunlight: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Promethazine Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Perform the test under the protection from sunlight. Dissolve 0.10 g of Promethazine Hydrochloride in exactly 5 mL of ethanol (95) and use this solution as the test solution. Pipet 1 mL of the test solution, add ethanol (95) to make exactly 200 mL and use this solution as the standard solution (1). Separately, dissolve 20 mg of isopromethazine hydrochloride RS in ethanol (95) to make exactly 100 mL and use this solution as the standard solution (2). Perform the test with the test solution and the standard solutions (1) and (2) as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solutions (1) and (2) on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol and diethylamine (19 : 1) to a distance of about 12 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots from the test solution corresponding to the spots from the standard solution (2) are not more intense than the spot from the standard solution (2) and any spot other than the principal spot from the test solution is not more intense than the spot from the standard solution (1).

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

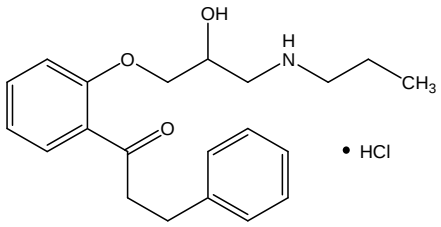
Assay Weigh accurately about 0.5 g of Promethazine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.088 mg of $C_{17}H_{20}N_2S \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Propafenone Hydrochloride



$C_{21}H_{27}NO_3 \cdot HCl$: 377.91

1-{2-[2-Hydroxy-3-(propylamino)propoxy]phenyl}-3-phenylpropan-1-one hydrochloride [*14222-60-7*]

Propafenone Hydrochloride contains not less than 98.0 % and not more than 102.0 % of propafenone hydrochloride ($C_{21}H_{27}NO_3 \cdot HCl$), calculated on the dried basis.

Description Propafenone Hydrochloride appears as white powder.

Propafenone Hydrochloride is soluble in methanol or in hot water, slightly soluble in ethanol (95) or in chloroform, very slightly soluble in acetone and practically insoluble in ether or in toluene.

Identification (1) Determine the infrared spectra of Propafenone Hydrochloride and Propafenone Hydrochloride RS, as directed in the potassiumn bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Dissolve 0.5 g of Propafenone Hydrochloride in 50 mL of water by heating and adjust with 0.1 mol/L sodium hydroxide TS to a pH of 9.5 to 10.0: a precipitate is formed. Cool the mixture, and filter. Add 1 mL of 6 mol/L nitric acid TS and 2 to 3 drops of 0.1 mol/L silver nitrate TS to the filtrate: a precipitate is formed. It dissolves upon the addition of 2 to 3 drops of ammonia solution (28).

Melting Point 171 ~ 175 °C.

pH Dissolve 0.5 g of Propafenone Hydrochloride in 100 mL of water: the pH of this solution is between 5.0 and 6.2.

Purity (1) ***Clarity of solution***—Dissolve 1.0 g of Propafenone Hydrochloride in 30 mL of hot water: the solution is clear.

(2) ***Heavy metals***—Proceed with 1.0 g of Propafenone Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) ***Related substances***—Weigh accurately about 0.10 g of Propafenone Hydrochloride, dissolve in 20 mL of the mobile phase from the operating conditions 1, and use this solution as the test solution. Pipet 2 mL of this solution, dissolve in 50 mL of the mobile phase from the operating conditions 1, pipet 2.5 mL of this solution, add 2.5 mL of a solution of diphenylphthalate in methanol (1 in 2000) and the mobile phase from the operating conditions 1 to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the operating conditions 1 and according to the operating conditions 2, and determine each peak area of the test solution by the automatic integration method. The area of any peak other than propafenone from the test solution is not larger than the peak area of propafenone from the standard solution.

Operating conditions 1

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: Dissolve 4.6 g of sodium 1-nonanesulfonate and 2.3 g of phosphoric acid in water to make exactly 1000 mL, and filter through a membrane filter with a pore size of 0.45 μm. To 900 mL of this solution add 600 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of diphenylphthalate is about 39 minutes.

System suitability 1

System performance: Dissolve 12 mg of propafenone hydrochloride and 50 mg of isopropyl benzoate in 100 mL of methanol. When the procedure is run with 10 μL of this solution under the above operating conditions, propafenone and isopropyl benzoate are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of propafenone is not more than 2.0 %.

Time span of measurement: As long as the retention time of diphenylphthalate, beginning after the solvent peak.

Operating conditions 2

Detector, column, and column temperature: Proceed as directed in the operating conditions 1.

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: Dissolve 7.33 g of sodium 1-nonanesulfonate and 2.3 g of phosphoric acid in water to make exactly 1000 mL, and filter through a membrane filter with a pore size of 0.45 μm. To 700 mL of this solution add 700 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of diphenylphthalate is about 11 minutes.

System suitability 2

System performance: Dissolve 12 mg of propafenone hydrochloride and 50 mg of isopropyl benzoate in 100 mL of methanol. When the procedure is run with 10 μL of this solution under the above operating conditions, propafenone and isopropyl benzoate are eluted in this order with the resolution between these peaks being not less than 21.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of propafenone is not more than 2.0 %.

Time span of measurement: About 2.5 times as long as the retention time of diphenylphthalate, beginning after the solvent peak.

Loss on Drying Not more than 0.5 % (1 g, 105 °C,

constant mass).

Residue on Ignition Not more than 0.1 % (1 g).

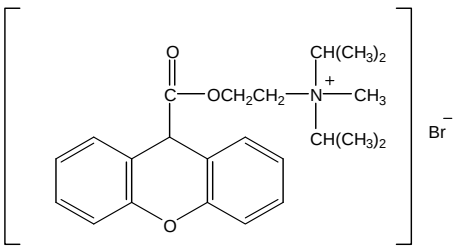
Assay Weigh accurately about 0.25 g of Propafenone Hydrochloride, dissolve in 30 mL of methanol, add 15 mL of mercuric acetate, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 37.79 mg of $C_{21}H_{27}NO_3 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Propantheline Bromide



$C_{23}H_{30}BrNO_3$: 448.39

N-Isopropyl-*N*-methyl-*N*-{2-[(9*H*-xanthen-9-ylcarbonyl)oxy]ethyl}propan-2-aminium bromide [*50-34-0*]

Propantheline Bromide, when dried, contains not less than 98.0 % and not more than 102.0 % of propantheline bromide ($C_{23}H_{30}BrNO_3$).

Description Propantheline Bromide is a white to yellowish white, crystalline powder, is odorless and has a very bitter taste.

Propantheline Bromide is very soluble in water, in acetic acid (100), in ethanol (95), or in chloroform, soluble in acetic anhydride and practically insoluble in ether.

pH—The pH of a solution of Propantheline Bromide (1 in 50) is between 5.0 and 6.0.

Melting point—About 161 °C (with decomposition, after drying).

Identification (1) Take 5 mL of a solution of Propantheline Bromide (1 in 20), add 10 mL of sodium hydroxide TS, heat to boil for 2 minutes. Cool to 60 °C and add 5 mL of dilute hydrochloric acid. After cooling, collect the precipitates and wash with water. Recrystallize from dilute ethanol and dry at 105 °C for 1 hour: the crystals melt between 217 °C and 222 °C.

(2) Dissolve 10 mg of the crystals obtained in (1) in 5 mL of sulfuric acid: a vivid yellow to yellow-red color develops.

(3) Take 5 mL of a solution of Propantheline Bromide (1 in 10), add 2 mL of dilute nitric acid: this solution responds to the Qualitative Tests (1) for bromide.

Purity ***Related substances***—Weigh accurately about 60 mg of Propantheline Bromide and dissolve in the mobile phase to make exactly 200 mL and use this solution as the test solution. Separately, weigh accurately a portion of each 9-bromohydroxypropantheline RS, xanthantanoic acid RS and xanthon RS, add mobile phase to make the solution to contain 6.0 μg of 9-bromo-hydroxy-propantheline, 1.5 μg each of xanthantanoic acid and xanthon per mL and use this solution as the standard solution. Perform the test with each 50 μL of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine the peak areas, A_T and A_S, for the test solution and the standard solution, respectively: xanthantinoic acid and xanthone are not more than 0.5 % and 9-bromohydroxyproprntheline is not more than 2.0 %. the total peak area of peaks of not less than 0.1 % except the solvent peak and the principal peak is not more than 3.0 %.

$$\text{Amount (mg) of related substance} = 100 \times \frac{C}{C_x} \times \frac{A_T}{A_S}$$

C: Concentration (μg/mL) of each related substance in the standard solution.

C_x: Concentration (μg/mL) of propantheline bromide in the test solution.

$$\text{Amount (\%) of unidentified related substance, not less than 0.1 \%} = 100 \times \frac{A_i}{A_t}$$

A_i: Peak area of an unidentified peak

A_t: Peak area of all peaks

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Mobile phase: A mixture of acetonitrile and pH 3.5 buffer (55 : 45).

Flow rate: 2.0 mL/minute.

System suitability

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the resolution between the least resolved peaks is not less than 1.2.

System repeatability: When the test is repeated 6 times with 50 μL each of the standard solution as directed under the above operating conditions, the relative standard deviation of each peak areas is not more

than 6.0 %.

pH 3.5 buffer solution—Prepare as directed in the Assay under Propantheline Bromide Tablets.

Loss on Drying Not more than 0.5 % (2 g, 105 °C, 4 hours).

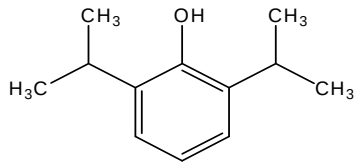
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 1.0 g of Propantheline Bromide, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 44.84 mg of $C_{23}H_{30}BrNO_3$.

Containers and Storage ***Containers***—Well-closed containers.

Propofol



$C_{12}H_{18}O$: 178.27

2,6-Diisopropylphenol [*2078-54-8*]

Propofol contains not less than 98.0 % and not more than 102.0 % of propofol ($C_{12}H_{18}O$).

Description Propofol is a colorless or pale yellow, clear liquid.
Propopol is very soluble in methanol or in ethanol (95), slightly soluble in cyclohexane or in 2-propanol, and very slightly soluble in water.

Identification Determine the infrared spectra of Propopol and Propopol RS, as directed in the liquid film method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive index n_D^{20} : 1.5125 ~ 1.5145.

Purity (1) ***Related substance I***—Dissolve 0.5 g of Propofol, accurately weighed, in hexane to make exactly 10 mL and use this solution as the test solution. Separately, dissolve 5 mg of propofol related substance I {2,6-*bis*(1-methylethyl)-1,4-benzoquinone}, accurately weighed, in hexane to make exactly 100 mL and use this solution as the standard solution. Perfom the test with 20 μL each of the test solution and the standard solution according to the operating conditions in the Assay as directed under Liquid Chromatography: the area of the related substance I peak from the test solution is not more than 5 times the area of the the related substance I peak from the standard solution.

Time span of measurement: About 6 times as long as the retention time of propopol.

(2) ***Related substances***—Dissolve 1.0 g of Propofol, accurately weighed, in hexane to make exactly 10 mL and use this solution as the test solution. Pipet 1.0 mL of the test solution, dilute with hexane to make exactly 100 mL. Pipet 1.0 mL of this solution, dilute with hexane to make exactly 10 mL and use this solution as the standard solution. Perfom the test with 10 μL each of the test solution and the standard solution according to the operating conditions in the Assay as directed under Liquid Chromatography: the area of the peak due to propopol related substance II {2-(1-methylethoxy)-1,3-*bis*(1-methylethyl)
benzene} is not more than 0.4 times the area of the peak due to propopol obtained from the standard solution (0.2 % taking into account a response factor of 0.2), and the area of the peak due to propopol related substance III {3,3',5,5'-tetrakis(1-methylethyl) biphenyl-4-4'-diol} is not more than 0.4 times the area of the peak due to propopol obtained from the standard solution (0.01 % taking into account a response factor of 4.0). The area of any other peak except peaks of propopol and the two related substances is not more than 0.5 times the area of the peak due to propopol obtained from the standard solution (0.5 %). The sum of all related substances including the related substance II and the related substance III is not more than 0.3 %. Disregard any peak with an area less than 0.25 times the area of the peak due to propopol obtained from the standard solution.

Assay Dissolve about 0.24 g of Propopol, accurately weighed, in hexane to make exactly 100 mL and use this solution as the test solution. Separately, dissolve about 0.24 g of Propopol RS, accurately weighed, in hexane to make exactly 100 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and measure the areas of propopol obtained from each solutions, A_T and A_S.

$$\text{Amount (mg) of propopol } (C_{12}H_{18}O) = \text{Amount (mg) of Propopol RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer

(wavelength: 275 nm).

Column: A stainless steel column about 5 mm in internal diameter and about 20 cm in length, packed with silica gel for liquid chromatography (50 μm in particle diameter).

Mobile phase: A mixture of hexane, acetonitrile, and ethanol (95) (990 : 7.5 : 1)

Flow rate: 2.0 mL/minute.

System suitability

System performance: When the procedure is run with 10 μL of the system suitability solution (1) under the above operating conditions, the related substance I (the retention time is about 2.5 minutes) and the propopol are eluted in this order, and the resolution between them is not less than 4.0. When the procedure is run with 10 μL of the system suitability solution (2) under the above operating conditions, the relative retention times of peaks due to the related substance II and the related substance III to that of propopol are about 0.5 and about 5, respectively.

Time span of measurement: About 5 times as long as the retention time of propopol.

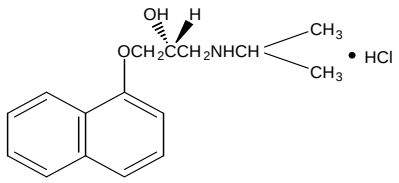
System suitability solution (1)—Dissolve 5 mg of Propopol RS and 15 mg of the related substance I RS in hexane to make 15 mL.

System suitability solution (2)—Dissolve 1.0 g of Propopol RS in hexane to make 10 mL.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and filled with inert gas.

Propranolol Hydrochloride



$C_{16}H_{21}NO_2 \cdot HCl$: 295.80

(*RS*)-1-(Isopropylamino)-3-(naphthalen-1-yloxy)propan-2-ol hydrochloride [*318-98-9*]

Propranolol Hydrochloride, when dried, contains not less than 99.0 % and not more than 101.0 % of propranolol hydrochloride ($C_{16}H_{21}NO_2 \cdot HCl$).

Description Propranolol Hydrochloride is a white, crystalline powder, is odorless and has a bitter taste.
Propranolol Hydrochloride is freely soluble in methanol, soluble in water, in acetic acid (100), or in ethanol (95), sparingly soluble in chloroform and practically insoluble in ether.
Propranolol Hydrochloride is colored by light.

Identification (1) Determine the absorption spectra of solutions of Propranolol Hydrochloride and Propranolol Hydrochloride RS in methanol (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Propranolol Hydrochloride and Propranolol Hydrochloride RS, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Propranolol Hydrochloride (1 in 50) responds to the Qualitative Tests (2) for chloride.

Melting Point 163 ~ 166 °C

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Propranolol Hydrochloride in 20 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Propranolol Hydrochloride according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Dissolve 20 mg of Propranolol Hydrochloride in 10 mL of the mobile phase, and use this solution as the test solution. Pipet 2 mL of the test solution, and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 ml, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the test solution and the standard solution as directed under Liquid Chromatorraphy according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than propranolol is not larger than 1/2 times the peak area of propranolol from the standard solution, and the total area of the peaks other than the peak of propranolol is not larger than 2 times the peak area of propranolol from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 292 jmm).

Column: A stainless steel column 4.6 mm in internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter)

Column temperature: A constant temperature of about 25 °C.

Mobile phase: Dissolve 1.6 g of sodium lauryl sulfate and 0.31 g of tetrabutylammonium phosphate in 450 mL of water, add 1 mL of sulfuric acid and 550 mL of acetonitrile for liquid chromatography, and adjust to pH 3.3 with 2 mol/L sodium hydroxide TS.

Flow rate: Adjust the flow rate so that the retention time of propranolol is about 4 minutes.

System suitability

Test for required detectability: Measure exactly 5 mL of the standard solution, and add the mobile phase

to make exactly 20 mL. Confirm that the peak area of propranolol obtained with 20 μL of this solution is equivalent to 17 to 33 % of that with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of propranolol is not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of propranolol is not more than 2.0 %.

Time span of measurement: About 5 times as long as the retention time of propranolol.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

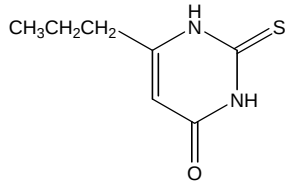
Assay Weigh accurately about 0.5 g of Propranolol Hydrochloride, previously dried, dissolve in 20 mL of acetic acid (100), add 30 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.580 mg of $C_6H_{21}NO_2 \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Propylthiouracil



$C_7H_{10}N_2OS$: 170.23

6-Propyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one
[*51-52-5*]

Propylthiouracil, when dried, contains not less than 98.0 % and not more than 101.0 % of propylthiouracil ($C_7H_{10}N_2OS$).

Description Propylthiouracil appears as white powder, odorless and has a bitter taste.
Propylthiouracil is sparingly soluble in ethanol (95) and very slightly soluble in water or in ether.
Propylthiouracil dissolves in sodium hydroxide TS or in ammonia TS.

Identification (1) Shake well 20 mg of Propylthiouracil with 7 mL of bromine TS for 1 minute and heat until the color of bromine TS disappears. Cool, filter and add 10 mL of barium hydroxide TS to the filtrate: a white precipitate is produced. The color of the precipitate does not turn purple within 1 minute.

(2) Take 5 mL of a hot saturated solution of Propylthiouracil, add 2 mL of a solution of sodium pentacyanoammine ferroate (1 in 100): a green color develops.

Melting Point 218 ~ 221 °C.

Purity (1) ***Sulfate***—Triturate Propylthiouracil to the finely powder, to 0.75 g of the powder, add 25 mL of water, heat for 10 minutes in a water-bath, cool, filter and wash the residue with water until the volume of the filtrate becomes 30 mL. To 10 mL of the filtrate, add 1 mL of dilute hydrochloric acid and water to make 50 mL and perform the test with this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.077 %).

(2) ***Heavy metals***—Proceed with 1.0 g of Propylthiouracil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Selenium***—Proceed with 0.2 g of Propylthiouracil, accurately weighed, as directed under Oxygen Flask Combustion Method, using 25 mL of diluted nitric acid (1 in 30) as the absorbing liquid. Use a 1 L combustion flask. After combustion, wash the stopper and the inside of the flask with 10 mL of water. Transfer the liquid inside the flask to a 150 mL beaker, using about 20 mL of water. Heat gently to boil, boil for 10 minutes, cool to room temperature, and use this solution as the test solution. Separately, pipet 6.0 mL of selenium standard stock solution, add 25 mL of diluted nitric acid (1 in 30) and 25 mL of water, and use this solution as the standard solution. Adjust the pH of the test solution and standard solution to 2.0 ± 0.2 with diluted ammonia solution (28) (1 in 2), add water to make exactly 60 mL, transfer to a separatory funnel using 10 mL of water, and wash the separatory funnel with 10 mL of water. Add 0.2 g of hydroxylamine hydrochloride, stir to dissolve, add immediately 5.0 mL of 2,3-diaminonaphthalene TS, stopper, stir to mix, and allow to stand at room temperature for 100 minutes. Add 5.0 mL of cyclohexane, shake vigorously for 2 minutes, allow the layers to separate, discard the water layer, centrifuge the cyclohexane extract to remove water, and take the cyclohexane layer. Determine the absorbances at 380 nm of these solutions as directed under Ultraviolet-visible Spectrophotometry, using a solution prepared by adding 25 mL of water to 25 mL of diluted nitric acid (1 in 30) and proceeding in the same manner, as the blank: the absorbance obtained

from the test solution is not more than that from the standard solution (not more than 30 ppm).

(4) ***Related substances***—Weigh accurately about 100 mg of Propylthiouracil, dissolve in methanol to make exactly 10 mL, and use this solution as the test solution. Separately, weigh accurately 10 mg of Propylthiouracil RS, dissolve in methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of toluene, ethyl acetate, and formic acid (10 : 9 : 1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm and 365 nm): any spot other than the principal spot obtained from the test solution is not larger or more intense than the spot from the standard solution (not more than 2.0 %). Disregard any spot remaining at the origin line.

(5) ***Thiourea***—Dissolve 0.3 g of Propylthiouracil in 50 mL of water by heating under a reflux condenser for 5 minutes, cool and filter. To 10 mL of the filtrate, add 3 mL of ammonia TS, shake well and add 2 mL of silver nitrate TS: the solution has no more color than the following control solution.

Control solution—Weigh exactly 60 mg of thiourea and dissolve in water to make exactly 100 mL. Pipet 1.0 mL of this solution, add water to make exactly 100 mL and proceed with 10 mL of this solution in the same manner.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.3 g of Propylthiouracil, previously dried and add 30 mL of water. Add 30 mL of 0.1 mol/L sodium hydroxide VS, heat to boil and dissolve by stirring. Wash down the solid adhering to the wall of the flask with a small volume of water and add 50 mL of 0.1 mol/L silver nitrate VS with stirring. Boil gently for 5 minutes, add 1 to 2 mL of bromothymol blue TS and titrate with 0.1 mol/L sodium hydroxide VS until a persistent blue-green color develops. Determine the total volume of 0.1 mol/L sodium hydroxide VS consumed.

Each mL of 0.1 mol/L sodium hydroxide VS
= 8.512 mg of $C_7H_{10}N_2OS$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Propylthiouracil Tablets

Propylthiouracil Tablets contain not less than 93.0 % and not more than 107.0 % of the labeled amount of propylthiouracil ($C_7H_{10}N_2OS$: 170.23).

Method of Preparation Prepare as directed under Tablets, with Propylthiouracil.

Identification Take a portion of powdered Propylthiouracil Tablets, equivalent to 0.3 g of Propylthiouracil according to the labeled amount, add 5 mL of ammonia TS, allow to stand for 5 minutes with occasional shaking, add 10 mL of water and centrifuge. To the clear supernatant liquid, add acetic acid, collect the precipitate produced, recrystallize from water and dry at 105 °C for 1 hour: it melts between 218 °C and 221 °C. Proceed with the residue as directed in the Identification under Propylthiouracil.

Dissolution Test Perform the test with 1 tablet of Propylthiouracil Tablets at 75 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of the 2nd fluid for dissolution test as the dissolution solution. Take 20 mL or more of the dissolved solution after 30 minutes from the starting of the test and filter through a membrane filter with a pore size of not more than 0.8 μm. Discard the first 10 mL of the filtrate and use the subsequent filtrate as the test solution. Separately, weigh accurately about 50 mg of Propylthiouracil RS, previously dried at 105 °C for 3 hours, dissolve in the 2nd fluid for dissolution test to make exactly 1000 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 274 nm as directed under Ultraviolet-visible Spectrophotometry.
The dissolution rate of Propylthiouracil Tablets in 30 minutes is not less than 80 %.

Dissolution rate (%) with respect to the labeled amount of propylthiouracil ($C_7H_{10}N_2OS$) = $W_S \times \frac{A_T}{A_S} \times \frac{1}{C} \times 90$

W_S : Amount (mg) of Propylthiouracil RS,

C: Labeled amount (mg) of propylthiouracil ($C_7H_{10}N_2OS$) in 1 tablet.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Propylthiouracil Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of propylthiouracil ($C_7H_{10}N_2OS$), add 150 mL of the 2nd fluid for dissolution test, sonicate to disperse into small particles, and add the 2nd fluid for dissolution test to make exactly 200 mL. Filter through a membrane filter

with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add the 2nd fluid for dissolution test to make exactly 100 mL, and use this solution as the test solution. Separately, weigh accurately about 50 mg of Propylthiouracil RS, previously dried at 105 °C for 2 hours, and dissolve in the 2nd fluid for dissolution test to make exactly 200 mL. Pipet 2 mL of this solution, add the 2nd fluid for dissolution test to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 274 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of propylthiouracil } (C_7H_{10}N_2OS) = \text{Amount (mg) of Propylthiouracil RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Protamine Sulfate

Protamine Sulfate is the sulfate of protamine prepared from the mature spermary of fish belonging to the family *Salmonidae* and others.

Protamine Sulfate has the property of binding to heparin. Protamine Sulfate binds to not less than 100 units of heparin sodium per mg of protamine sulfate, calculated on the dried basis.

Description Protamine Sulfate is a white to pale grayish yellow powder.

Protamine Sulfate is slightly soluble in water and practically insoluble in ethanol (95) or in ether.

Identification (1) Dissolve 1 mg of Protamine Sulfate in 2 mL of water, add 5 drops of a solution prepared by dissolving 0.1 g of 1-naphthol in 100 mL of diluted ethanol (7 in 10) and 5 drops of sodium hypochlorite TS, then add sodium hydroxide TS until the solution becomes alkaline: a vivid red color develops.

(2) Dissolve 5 mg of Protamine Sulfate in 1 mL of water by warming, add 1 drop of a solution of sodium hydroxide (1 in 10) and 2 drops of copper (II) sulfate TS: a red-purple color develops.

(3) The solution of Protamine Sulfate (1 in 20) responds to the Qualitative Tests for sulfate.

pH Dissolve 1.0 g of Protamine Sulfate in 100 mL of water: the pH of this solution is between 6.5 and 7.5.

Absorbance Determine the absorbance of a 1.0 % solution of Protamine Sulfate as directed under Ultraviolet-visible Spectrophotometry, using water as the blank: the difference in absorbance between 260 nm and 280 nm is not more than 0.1.

Purity (1) ***Clarity and color of solution***—Dissolve 0.10 g of Protamine Sulfate in 10 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—To 1.0 g of Protamine Sulfate add 0.5 g of magnesium oxide, mix, and carbonize. After cooling, ignite to incinerate at a temperature not exceeding 800 °C for 1 hour. After cooling, dissolve the residue in 5 mL of a mixture of hydrochloric acid and water (1 : 1). Add 0.1 mL of phenolphthalein TS, then add ammonia solution (28) dropwise until the solution develops a pale red color. After cooling, add acetic acid (100) until the color disappears and add a further 0.5 mL. Filter if necessary, and wash. Add water to make 20 mL, and use this solution as the test solution. Separately, proceed with 2.0 mL of standard lead solution instead of Protamine Sulfate in the same manner as the test solution. To 10 mL of this solution add 2 mL of the test solution, and use this solution as the control solution. Separately, to 10 mL of water add 2 mL of the test solution, and use this solution as the blank solution. To 12 mL each of the test solution, control solution, and blank solution add 2 mL of pH 3.5 acetic acid buffer solution, mix, add 1.2 mL of thioacetamide TS, and mix immediately. Allow to stand for 2 minutes: the color of the test solution is not more intense than that of the control solution (not more than 20 ppm).

System suitability: The control solution shows a slightly brown color compared to the blank solution. To the test solution add 2.0 mL of standard lead solution, to 10 mL of this solution add 2 mL of the test solution, and use this solution as the system suitability solution. The system suitability solution is not less intense than the control solution.

(3) ***Mercury***—Transfer 2.0 g of Protamine Sulfate to a glass-stoppered conical flask, and add 20 mL of a mixture of nitric acid and sulfuric acid (1 : 1). Boil with a reflux condenser for 1 hour. After cooling, carefully add water to dilute. Boil again until nitric acid fumes are no longer evolved. After cooling, carefully add water to make 200 mL, and filter. Transfer 50 mL of the filtrate to a separatory funnel, and extract several times with a small volume of chloroform until the chloroform layer is colorless. Discard the chloroform layer. To the water layer add 25 mL of dilute sulfuric acid, 115 mL of water, and 10 mL of a solution of hydroxylamine hydrochloride (2 in 10), and titrate with dithizone solution while shaking vigorously until the color of the mixture changes to blue-green (not more than 10 ppm).

1 mL of dithizone solution = 1 μg of Hg

Dithizone solution—Dissolve 40.0 mg of dithizone in 1000 mL of chloroform. Pipet 30.0 mL of this solution, and add chloroform to make 100 mL.

Loss on Drying Not more than 5.0 % (1 g, 105 °C, 3 hours).

Abnormal Toxicity Dissolve 0.5 of Protamine Sulfate in 0.5 mL of water for injection, and inject intravenously for 15 to 30 seconds to each of 5 healthy mice weighing 17 to 24 g. Use animals in which no abnormalities are observed for not less than 5 days prior to the test. No animals die during the 24 hour post-dosage observation. If 1 animal dies, repeat the test with 5 animals: no animals die during the 24 hour observation.

Nitrogen Content Weigh accurately about 10 mg of Protamine Sulfate, and proceed as directed under Nitrogen Determination: the amount of nitrogen (N: 14.01) is 22.5 to 25.5 %, calculated on the dried basis.

Sulfate Weigh accurately about 0.150 g of Protamine Sulfate, dissolve in 75 mL of water, add 5 mL of 3 mol/L hydrochloric acid TS, and slowly add 10 mL of barium chloride TS while boiling. Close the lid, allow to stand in a steam bath for 1 hour, and filter. Wash the residue several times with ho twater, dry, and ignite to constant mass. Multiply the amount of the residue thus obtained by 0.4117 and calculate the amount of sulfate: 16 to 22 %, calculated on the dried basis.

Heparin-Binding Capacity (1) ***Test solution A***—Weigh accurately 15 mg of Protamine Sulfate, dissolve in water to make exactly 100 mL, and use this solution as the test solution. Repeat this procedure 3 times, and use the solutions so obtained as test solutions (A1), (A2), and (A3).

(2) ***Test solution B***—Pipet 10 mL each of test solutions (A1), (A2), and (A3), add 5 mL of water to each, and use these solutions as test solutions (B1), (B2), and (B3).

(3) ***Test solution C***—Pipet 10 mL each of test solutions (A1), (A2), and (A3), add 20 mL of water to each, and use these solutions as test solutions (C1), (C2), and (C3).

(4) ***Heparin sodium standard solution***—Weigh accurately an amount of Heparin Sodium RS, dissolve in water so that each mL contains 20 units, and use this solution as the standard solution.

(5) ***Procedure***—Pipet 2 mL of the test solution, transfer to a spectrophotometer cell, add the heparin sodium standard solution dropwise while mixing, and determine the transmittance at 500 nm as directed under Ultraviolet-visible Spectrophotometry. Calculate the amount of the standard solution consumed, V mL, until a sharp change in transmittance is observed. Perform the test a total of 18 times, repeating 2 times for each test solution.

(6) ***Calculation***—Calculate the amount of heparin bound with 1 mg of Protamine Sulfate by the following equation, using the amount (mL) of the standard solution consumed obtained from each test solution, and calculate the average of all 18 tests. The test is not valid unless each relative standard deviation of the 6 results obtained from test solutions A, B, and C is not more than 5 %, respectively, and also unless each relative standard deviation of the 6 results obtained from the test solution sets (A1, B1, C1), (A2, B2, C2), and (A3, B3, C3) is not more than 5 %, respectively.

$$\text{Amount (heparin units) of heparin bound to 1 mg of Protamine Sulfate} = \text{Concentration (heparin units/mL) of the standard solution} \times V \times \frac{50}{W_T} \times d$$

W_T: Amount (mg) of the sample, calculated on the anhydrous basis.

d: Dilution factor (A) of each solution with respect to test solution.

Containers and Storage ***Containers***—Tight containers.

Protamine Sulfate Injection

Protamine Sulfate Injection is an aqueous solution for injection.

Protamine Sulfate Injection contains not less than 92.0 % and not more than 108.0 % of the labeled amount of protamine sulfate. Protamine Sulfate Injection binds to not less than 100 units of heparin sodium per mg of protamine sulfate.

Method of Preparation Prepare as directed under Injections, with Protamine Sulfate.

Description Protamine Sulfate Injection is a colorless liquid, is odorless or has the odor of preservatives.

Identification (1) Dilute a volume of Protamine Sulfate Injection, equivalent to 1 mg of protamine sulfate according to the labeled amount, with water to make 2 mL and proceed as directed in the Identification (1) under Protamine Sulfate.

(2) Dilute a volume of Protamine Sulfate Injection, equivalent to 5 mg of protamine sulfate according to the labeled amount, with water to make 1 mL and proceed as directed in the Identification (2) under Protamine Sulfate.

(3) Protamine Sulfate Injection responds to the Qualitative Tests for sulfate.

pH 5.0 ~ 7.0.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 6.0 EU/mg of protamine sulfate.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

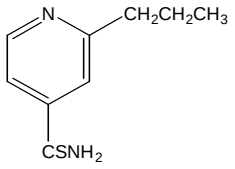
Determination of Volume of Injection in Containers It meets the requirement.

Assay (1) ***Protein***—Weigh accurately an amount of Protamine Sulfate Injection, equivalent to about 10 mg of Protamine Sulfate according to the labeled amount, transfer to a Kjeldahl flask, evaporate to dryness in a water bath with the aid of a current of air, and perform the test as directed under Nitrogen Determination. Calculate the amount of protein by converting 0.24 mg of nitrogen (N: 14.01) to 1 mg of protein.

(2) ***Heparin-binding capacity***—Proceed as directed in the Heparin-Binding Capacity under Protamine Sulfate, and calculate the amount of heparin sodium bound to each mL of protein. Prepare the test solution in (1) as follows: weigh accurately an amount of Protamine Sulfate Injection, equivalent to 15.0 mg of Protamine Sulfate according to the labeled amount, add water to make exactly 100 mL, and use this solution as the test solution. Repeat this procedure 3 times, and use the solutions so obtained as the test solutions (A1), (A2), and (A3). The amount of heparin sodium bound to 1 mg of the labeled amount of Protamine Sulfate Injection is not less than 100 units.

Containers and Storage ***Containers***—Hermetic containers.

Prothionamide



$C_9H_{12}N_2S$: 180.27

2-Propylpyridine-4-carbothioamide [*14222-60-7*]

Prothionamide, when dried, contains not less than 98.0 % and not more than 101.0 % of prothionamide ($C_9H_{12}N_2S$).

Description Prothionamide appears as yellow crystals or crystalline powder and has a slight, characteristic odor.
Prothionamide is freely soluble in methanol or in acetic acid (100), soluble in ethanol (95), slightly soluble in ether and practically insoluble in water.
Prothionamide dissolves in dilute hydrochloric acid or in dilute sulfuric acid.

Identification (1) Mix 50 mg of Prothionamide with 0.1 g of 1-chloro-2,4-dinitrobenzene, transfer about 10 mg of this mixture to a test tube and heat for several seconds over a small flame until the mixture is fused. Cool and add 3 mL of potassium hydroxide-ethanol TS: a red to orange color develops.

(2) Place 0.5 g of Prothionamide in a beaker and dissolve in 20 mL of sodium hydroxide TS by heating while shaking occasionally: the gas evolved turns a moistened red litmus paper to blue. Boil gently and evaporate the solution to 3 to 5 mL. After cooling, add gradually 20 mL of acetic acid (100) and heat in a water-bath: the gas evolved the moistened red lithmus paper to blue. Evaporate the solution in a water-bath to 3 mL to 5 mL with the aid of a current of air, cool, add 10 mL of water and mix well. Filter the crystals by suction, recrystallize from water immediately and dry in a desiccator (in vacuum, silica gel) for 6 hours: the crystals melt between 198 ºC and 203 ºC (with decomposition).

Melting Point 142 ~ 145 ºC.

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Prothionamide in 20 mL of ethanol (95): the solution is clear and shows a yellow color.

(2) ***Acid***—Dissolve 3.0 g of Prothionamide in 20 mL of methanol with warming. Add 100 mL of water to the solution, cool in an ice water-bath with agitation and remove any precipitate by filtration. Allow 80 mL of the filtrate to cool to room temperature and add 0.8 mL of cresol red TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(3) ***Heavy metals***—Proceed with 1.0 g of Prothionamide according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Arsenic***—Prepare the test solution with 0.6 g of Prothionamide according to Method 3 and perform the test. To the test solution, add 10 mL of a solution of magnesium nitrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30) and ignite to burn (not more than 3.3 ppm).

Loss on Drying Not more than 0.5 % (1 g, 80 ºC, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.3 g of Prothionamide, previously dried, dissolve in 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from orange to dark orange-brown (indicator: 2 mL of 1-naphtholbenzeine TS). Perform a blank determination and make any necessary correction.

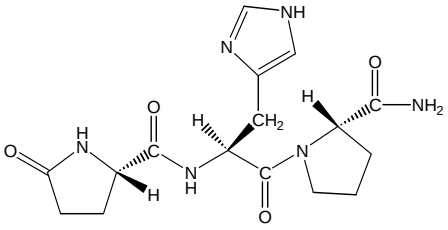
Each mL of 0.1 mol/L perchloric acid VS
= 18.027 mg of $C_9H_{12}N_2S$

Containers and Storage ***Containers***—Well-closed

containers.

Storage—Light-resistant.

Protirelin



$C_{16}H_{22}N_6O_4$: 362.38

(2*S*)-*N*-{(2*S*)-1-[(*S*)-2-Carbamoylpyrrolidin-1-yl]-3-(1*H*-imidazol-4-yl)-1-oxopropan-2-yl}-5-oxopyrrolidine-2-carboxamide [*24305-27-9*]

Protirelin contains not less than 98.5 % and not more than 101.0 % of protirelin ($C_{16}H_{22}N_6O_4$), calculated on the anhydrous basis.

Description Protirelin appears as white powder.
Protirelin is freely soluble in water, in methanol, in ethanol or in acetic acid (100).
Protirelin is hygroscopic.

Identification (1) Take 10 mg of Protirelin in a test tube made of hard glass, add 0.5 mL of 6 mol/L hydrochloric acid TS, seal the upper part of the tube and heat carefully at 110 ºC for 5 hours. After cooling, open the seal, transfer the contents into a beaker and evaporate on a water-bath to dryness. Dissolve the residue in 1 mL of water and use this solution as the test solution. Separately, dissolve 80 mg of L-glutamic acid, 0.12 g of L-histidine hydrochloride and 60 mg of L-proline in 20 mL of water and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, acetic acid (100) and pyridine (4 : 1 : 1 : 1) to a distance of about 12 cm and dry the plate at 100 ºC for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate and heat at 80º C for 5 minutes: the three spots obtained from the test solution show the same color and the same R_f value as each corresponding spots obtained from the standard solution.

(2) Determine the infrared spectra of Protirelin and Protirelin RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

pH Dissolve 0.2 g of Protirelin in 10 mL of water: the pH of this solution is between 7.5 and 8.5.

Specific Optical Rotation $[\alpha]_D^{20}$: -66.0 ~ -69.0º (calculated on the anhydrous basis, 0.1 g, water, 20 mL, 100 mm).

Purity (1) ***Clarity and color of solution***—Dissolve 0.1 g of Protirelin in 10 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Protirelin according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Dissolve 0.20 g of Protirelin in 10 mL of water and use this solution as the test solution. Pipet 1 mL of this solution, add water to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate (1) of silica gel for thin-layer chromatography and spot 5 μL of the test solution on a plate (2) of silica gel for thin-layer chromatography. Develop the plates with a mixture of 1-butanol, water, acetic acid (100) and pyridine (4 : 2 : 1 : 1) to a distance of about 12 cm and dry the plates at 100 ºC for 30 minutes. Spray evenly a mixture of a solution of sulfanilic acid in 1 mol/L hydrochloric acid TS (1 in 200) and a solution of sodium nitrite (1 in 20) (1 : 1) on the plate (1) and air-dry the plates. Successively spray evenly a solution of sodium carbonate (1 in 10): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate (2) and heat at 80 ºC for 5 minutes: no colored spot appears.

Water Not more than 5.0 % (0.1g, volumetric titration, direct titration).

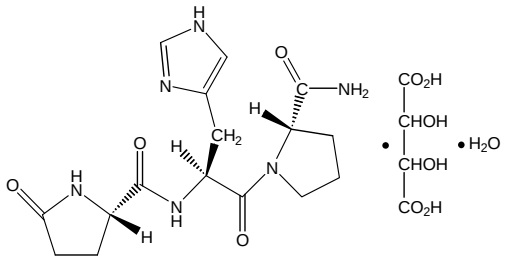
Residue on Ignition Not more than 0.3 % (0.2 g).

Assay Weigh accurately about 70 mg of Protirelin dissolve in 50 mL of acetic acid (100) and titrate with 0.02 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.02 mol/L perchloric acid VS
= 7.248 mg of $C_{16}H_{22}N_6O_4$

Containers and Storage ***Containers***—Tight containers.

Protirelin Tartrate Hydrate



$C_{16}H_{22}N_6O_4 \cdot C_4H_6O_6 \cdot H_2O$: 530. 49

(2*S*)-*N*-{(2*S*)-1-[(*S*)-2-Carbamoylpyrrolidin-1-yl]-3-(1*H*-imidazol-4-yl)-1-oxopropan-2-yl}-5-oxopyrrolidine-2-carboxamide (2*R*,3*R*)-2,3-dihydroxybutanedioate hydrate [*53935-32-3,* anhydride]

Protirelin Tartrate Hydrate contains not less than 98.5 % and not more than 101.0 % of protirelin tartrate ($C_{16}H_{22}N_6O_4 \cdot C_4H_6O_6$: 512.47), calculated on the anhydrous basis.

Description Protirelin Tartrate Hydrate appears as white to pale yellowish white crystals or crystalline powder.
Protirelin Tartrate Hydrate is freely soluble in water, sparingly soluble in acetic acid (100) and practically insoluble in ethanol (95) or in ether.

Melting point—About 187 °C (with decomposition).

Identification (1) Take 1 mL of a solution of Protirelin Tartrate Hydrate (1 in 1000), add 2 mL of a solution of 4-nitrobenzene diazonium fluoroborate (1 in 2000) and 2 mL of boric acid potassium chloride-sodium hydroxide buffer solution, pH 9.0: a red color develops.

(2) Dissolve 30 mg of Protirelin Tartrate Hydrate in 5 mL of sodium hydroxide TS, add 1 drop of copper (II) sulfate TS: a purple color develops.

(3) To 0.2 g of Protirelin Tartrate Hydrate, add 5.0 mL of 6 mol/L hydrochloric acid TS and boil for 7 hours under a reflux condenser. After cooling, evaporate 2.0 mL of this solution in a water-bath to dryness, dissolve the residue in 2.0 mL of water and use this solution as the test solution Separately, dissolve 22 mg of L-glutamic acid, 32 mg of L-histidine hydrochloride monohydrate and 17 mg of L-proline in 2.0 mL of 0.1 mol/L hydrochloric acid TS by heating and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 2 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, acetic acid (100) and pyridine (4 : 1 : 1 : 1) to a distance of about 12 cm and dry at 100 °C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate and dry at 80 °C for 5 minutes: the three spots obtained from the test solution show, respectively, the same color and the same R_f value as the corresponding spot from the standard solution.

(4) A solution of Protirelin Tartrate Hydrate (1 in 40) responds to the Qualitative Tests for tartrate.

Specific Optical Rotation $[\alpha]_D^{20}$: -50.0 ~ -53.0° (0.5 g, calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH Dissolve 1.0 g of Protirelin Tartrate Hydrate in 100 mL of water: the pH of this solution is between 3.0 and 4.0.

Purity (1) ***Clarity and color of solution***—Dissolve 0.10 g of Protirelin Tartrate Hydrate in 10 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Protirelin Tartrate Hydrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead aolution (not more than 20 ppm).

(3) ***Arsenic***—Take 1.0 g of Protirelin Tartrate Hydrate in a porcelain crucible. Add 10 mL of a solution of magnesium nitrate in ethanol (95) (1 in 10), ignite ethanol and heat gradually to incinerate. If a carbonized material still remains in this method, moisten with a small quantity of nitric acid and ignite to incinerate. After cooling, add 10 mL of dilute hydrochloric acid, heat in a waterbath to dissolve the residue, use this solution as the test solution and perform the test (not more than 2 ppm).

(4) ***Related Substances***—Dissolve 0.60 g of Protirelin Tartrate Hydrate in 10 mL of water and use this solution as the test solution. Pipet 1 mL of the test solution, add water to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate (1) of silica gel for thin-layer chromatography. Spot 5 μL of the test solution on a plate (2) of silica gel for thin-layer chromatography. Develop the plates with a mixture of chloroform, methanol and ammonia solution (28) (6 : 4 : 1) to a distance of about 10 cm and dry at 100 °C for 30 minutes. Spray evenly a mixture of a solution of sulfanilic acid in 1 mol/L hydrochloric acid TS (1 in 200) and a solution of sodium nitrite (1 in 20) (1:1) on the plate (1) and air-dry the plate. Then, spray evenly a solution of sodium carbonate (1 in 10) on the plate: the spots other than the principal spot from the test solution are not more intense than those from the standard solution in color. On the other hand, spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate (2) and dry at 80 °C for 5 minutes: no colored spot is obtained.

Water Not more than 4.5 % (0.2 g, volumetric titration, direct titration).

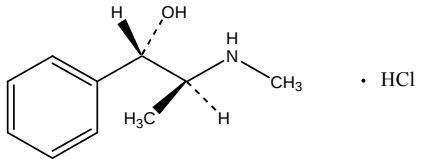
Residue on Ignition Not more than 0.2 % (0.5 g).

Assay Weigh accurately about 0.5 g of Protirelin Tartrate Hydrate, dissolve in 80 mL of acetic acid (100) by warming, cool and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 51.25 mg of $C_{16}H_{22}N_6O_4 \cdot C_4H_6O_6$

Containers and Storage ***Containers***—Well-closed containers.

Pseudoephedrine Hydrochloride



$C_{10}H_{15}NO \cdot HCl$: 201.69

(1*S*,2*S*)-2-(Methylamino)-1-phenylpropan-1-ol hydrochloride [*345-78-8*]

Pseudoephedrine Hydrochloride contains not less than 98.0 % and not more than 102.0 % of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$), calculated on the dried basis.

Description Pseudoephedrine Hydrochloride appears as fine white crystals or powder with a slightly characteristic ordor.
Pseudoephedrine Hydrochloride is very soluble in water, freely soluble in ethanol (95), and slightly insoluble in chloroform.

Identification (1) Determine the infrared spectra of Pseudoephedrine Hydrochloride and Pseudoephedrine Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) The solution of Pseudoephedrine Hydrochloride in water (1 in 50) responds to the Qualitative Tests for chloride (the Flame Coloration Test 2).

Specific Optical Rotation $[\alpha]_D^{20}$: +61.0 ~ +62.5° (0.5 g, water, 10 mL, 100 mm).

Melting Point 182 ~ 186 °C.

pH The pH of a solution obtained by dissolving 1.0 g of Pseudoephedrine Hydrochloride in 20 mL of water is between 4.6 and 6.0.

Purity ***Related substances***—Dissolve 0.1 g of Pseudoephedrine Hydrochloride, accurately weighed, in ethanol (95) to make exactly 10 mL, and use this solution as the test solution. Separately, dissolve 1.0, 5.0, 10.0 and 20.0 mg each of Pseudoephedrine Hydrochloride RS in ethanol (95) to make exactly 100 mL. Use these solutions as the standard solutions (1), (2), (3) and (4), respectively. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 20 μL each of the test solution and the standard solutions on a plate of silica gel with a fluorescent indicator for thin-layer chromatography, develop with a mixture of ethanol (95), acetic acid (100), and water (10 : 3 : 1) to a distance of about 15 cm, and dry the plate for 2 hours with a current from a hot air dryer. Expose the plate to iodine vapors for not less than 30 minutes. Compare the intensity of all the spots, excluding the principal spot, from the test solution with the intensity of the spots from the standard solutions: the sum of all related substances is not more than 2.0 %.

Loss on Drying Not more than 0.5 % (1 g, 105 ºC, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 100 mg of Pseudoephedrine Hydrochloride, transfer to a 100 mL volumetric flask, add water to make exactly 100 mL, mix, and use this solution as the test solution. If necessary, gradually dilute the concentration of the test solution to 0.1 mg/mL. Weigh accurately an amount of Pseudoephedrine Hydrochlordie RS, dissolve in water so that each mL contains 0.1 mg, and use this solution as standard solution (1). Separately, weigh accurately an amount each of Pseudoephedrine Hydrochloride RS and Ephedrine Sulfate RS, dissolve in water so that each mL contains 0.1 mg and 0.002 mg, respectively, and use this solution as standard solution (2). Perform the test with 10 μL each of the test solution and standard solution (1) as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of pseudoephedrine hydrochloride.

Amount (mg) of pseudoephedrine hydrochloride ($C_{10}H_{15}NO \cdot HCl$)
$$= 100 \times \frac{A_T}{A_S} \times \frac{C_S}{C_T}$$

C_S: Concentration (mg/mL) of pseudoephedrine in the standard solution

C_T: Concentration (mg/mL) of pseudoephedrine in the test solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 206 nm)

Column: A stainless steel column about 3.0 mm in internal diameter and about 15 cm in length, packed with phenyl silica gel for liquid chromatography (3.5 μm in particle diameter).

Mobile phase: Mix well 5 mL of triethylamine and 1000 mL of water, and adjust the pH to 6.8 with phosphoric acid. To 900 mL of this solution add 100 mL of water.

Flow rate: 0.6 mL/minute

System suitability

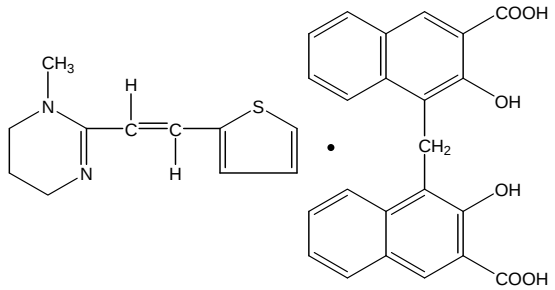
System performance: When the procedure is run with 10 μL of standard solution (2) under the above operating conditions, the relative retention times of ephedrine and pseudoephedrine are 0.9 and 1.0, respectively, with the resolution between these peaks being not less than 2. The symmetry factor of pseudoephedrine is not more than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL each of standard solution (2) under the above operating conditions, the relative standard deviation of the ratios of the peak area is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Pyrantel Pamoate



$C_{11}H_{14}N_2S \cdot C_{23}H_{16}O_6$: 594.68

4-[(3-Carboxy-2-hydroxynaphthalen-1-yl)methyl]-3-hydroxynaphthalene-2-carboxylic acid [*22204-24-6*]

Pyrantel Pamoate, when dried, contains not less than 98.0 % and not more than 101.0 % of pyrantel pamoate ($C_{11}H_{14}N_2S \cdot C_{23}H_{16}O_6$).

Description Pyrantel Pamoate is a pale yellow to yellow, crystalline powder, is odorless and tasteless.

Pyrantel Pamoate is sparingly soluble in *N,N*-dimethylformamide, very slightly soluble in methanol or ethanol (95), and practically insolubale in water, in ethyl acetate, in ether or in chloroform.

Melting point—256 ~ 264 °C (with decomposition).

Identification (1) Take 50 mg of Pyrantel Pamoate, add 10 mL of methanol and 1 mL of a mixture of hydrochloric acid and methanol (1 : 1) and shake vigorously: a yellow precipitate is produced. Filter the solution and use filtrate as the test solution, Use the precipitate for Identification (2). To 0.5 mL of the test solution, add 1 mL of a solution of 2,3-indoline dione in sulfuric acid (1 in 1000): a red color develops.

(2) Collect the precipitate obtained in (1), wash with methanol and dry at 105 °C for 1 hour. To 10 mg of the dried precipitate, add 10 mL of methanol, shake well and filter. To 5 mL of the filtrate, add 1 drop of iron (III) chloride TS: a green color develops.

(3) Dissolve 0.1 g each of Pyrantel Pamoate and Pyrantel Pamoate RS in 50 mL of *N,N*-dimethylformamide and add methanol to make 200 mL, respectively. To 2 mL each of these solutions, add a solution of hydrochloric acid in methanol (9 in 1000) to make 100 mL, respectively. Determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared spectra of Pyrantel Pamoate and Pyrante Pamotate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity (1) ***Chloride***—Take 1.0 g of Pyrantel Pamoate, add 10 mL of dilute nitric acid and 40 mL of water and heat on a water-bath with shaking for 5 minutes. After cooling, add water to make 50 mL and filter. To 20 mL of the filtrate, add 2 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036 %).

(2) ***Sulfate***—Take 0.75 g of Pyrantel Pamoate, add 5 mL of dilute hydrochloric acid and water to make 100 mL and heat on a water-bath for 5 minutes with shaking. After cooling, add water to make 100 mL and filter. To 20 mL of the filtrate, add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.45 mL of 0.005 mol/L sulfuric acid VS (not more than 0.144 %).

(3) ***Heavy metals***—Proceed with 1.0 g of Pyrantel Pamoate according to Method 2 and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

(4) ***Iron***—Weigh accurately 1.33 g of Pyrantel Pamoate, perform the Residue on Ignition Test, to the residue so obtained add 3 mL of hydrochloric acid and 2 mL of nitric acid, and evaporate to dryness in a steam bath. Dissolve this residue in 2 mL of hydrochloric acid with gentle heating. Add 18 mL of hydrochloric acid and water to make 50 mL. Pipet 5.0 mL of this solution, add water to make 47 mL, and use this solution as the test solution. Separately, to 1.0 mL of standard iron solution add water to make 45 mL and 2 mL of hydrochloric acid, and use this solution as the standard

solution. To the test solution and standard solution add 50 mg of ammonium peroxydisulfate and 3 mL of 30 % ammonium thiocyanate solution, and mix: the color obtained from the test solution is not more intense than that from the standard solution (not more than 75 ppm).

(5) ***Arsenic***—Prepare the test solution with 1.0 g of Pyrantel Pamoate according to Method 3 and perform the test (not more than 2 ppm).

(6) ***Related substances***—Perform the test under protection from direct sunlight in light-resistant vessels. Dissolve 0.10 g of Pyrantel Pamoate in l0 mL of *N,N*-dimethylformamide and use this solution as the test solution. Pipet 1 mL of the test solution, add *N,N*-dimethyl-formamide to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetic acid (100) and water (3 : 1 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the spot of pyrantel and pamoic acid from the test solution is not more intense than the spot of pyrantel (R_f value: about 0.3) from the standard solultion.

Loss on Drying Not more than 1.0 % (1 g, 105 °C, 2 hours)

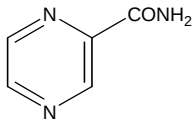
Residue on Ignition Not more than 0.3 % (1 g).

Assay Weigh accurately about 0.5 g of Pyrantel Pamoate, previously dried, add 25 mL of chloroform and 25 mL of sodium hydroxide TS, shake for 15 minutes and extract. Extract further with two 25 mL volumes of chloroform. Filter each extract through 5 g of anhydrous sodium sulfate on a pad of absorbent cotton. Combine the chloroform extracts, add 30 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (indicator: 2 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 59.47 mg of $C_{11}H_{14}N_2S \cdot C_{23}H_{16}O_6$

Containers and Storage ***Containers***—Tight containers.

Pyrazinamide



$C_5H_5N_3O$: 123.11

Pyrazine-2-carboxamide [*98-96-4*]

Pyrazinamide, when dried, contains not less than 99.0 % and not more than 101.0 % of pyrazinamide ($C_5H_5N_3O$).

Description Pyrazinamide appears as white crystals or crystalline powder, is odorless and has a slightly bitter taste.
Pyrazinamide is sparingly soluble in water, slightly soluble in ethanol (95) or acetic anhydride.

Identification (1) Determine the absorption spectra of solutions of Pyrazinamide and Pyrazinamide RS, respectively, in 0.1 mol/L hydrochloric acid TS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry : both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Pyrazinamide and Pyrazinamide RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 188 ~ 193 ºC.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Pyrazinamide according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Dissolve 0.10 g of Pyrazinamide in 10 mL of methanol, and use this solution as the test solution. Pipet 1 mL of the test solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 20 μL each of the test solution and standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3 : 1 : 1) to a distance of about 10cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot from the test solution is not more intense than the spot from the standard solution .

Loss on Drying Not more than 0.5 % (1 g, in vacuum, silica gel, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.1 g of Pyrazinamide, previously dried, in 50 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 12.31 mg of $C_5H_5N_3O$

Containers and Storage ***Containers***—Well-closed containers.

Pyrazinamide Tablets

Pyrazinamide Tablets contain not less than 93.0 % and not more than 107.0 % of the labeled amount of pyrazinamide ($C_5H_5N_3O$: 123.11).

Method of Preparation Prepare as directed under Tablets, with Pyrazinamide.

Identification (1) Take a portion of powdered Pyrazinamide Tablets, equivalent to about 1 g of Pyrazinamide, add about 75 mL of isopropanol, heat in the water-bath and filter when it is hot. Allow to cool, filter the crystals that form and dry at 105 ºC for 1 hour. Determine the absorption spectra of the aqueous solutions (1 in 100000) of the crystals so obtained and Pyrazinamide RS, respectively, under Ultraviolet-visible Spectrophotometry: both spectra exhibit maximum and minimum at the same wavenumbers. Determine the absorption spectra of the crystal solution and Pyrazinamide RS at the wavelength of maximum absorption at about 268 nm, respectively: the difference is not more than 3.0 %.

(2) Take 20 mg of the crystals obtained in Identification (1), add 5 mL of 5 mol/L sodium hydroxide and heat gently: the odor of ammonia is perceptible.

Dissolution Test Perform the test with 1 tablet of Pyrazinamide Tablets at 50 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of water as the dissolution solution. Take the dissolved solution after 45 minutes from start of the test, filter, dilute with water, if necessary and use this solution as the test solution. Separately, weigh accurately a portion of Pyrazinamide RS, add water to make constant concentration and use this solution as the standard solution. Determine the absorbances of the test solution and the standard solution at 268 nm as directed under Ultraviolet-visible Spectrophotometry using the water as the blank.
The dissolution rate of Pyrazinamide Tablets in 45 minutes is not less than 75 %.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Pyrazinamide Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of pyrazinamide ($C_5H_5N_3O$) and transfer with the aid of 300 mL of water to a volumetric flask. After sonication for 10 minutes, add water to make exactly 500 mL and mix. Filter this solution, discard the first portion of the filtrate, take exactly 20 mL of the subsequent filtrate, add water to make 100 mL and use this solution as the test solution. Separately, weigh accurately about 0.1 g of Pyrazinamide RS, add water to make exactly 500 mL, pipet 20 mL of this solution, add water to make exactly 100 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the peak areas, A_T and A_S, of pyrazinamide from the test solution and the standard solution, respectively.

$$\text{Amount (mg) of pyrazinamide } (C_5H_5N_3O) = \text{Amount (mg) of Pyrazinamide RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 270 nm)

Column: A stainless steel column, about 3.9 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 to 10 μm in particle diameter).

Mobile phase: Add phosphoric acid to phosphate buffer, pH 8.0 and adjust pH to 3.0. To 1000 mL of this solution add 10 mL of acetonitrile, shake and filter.

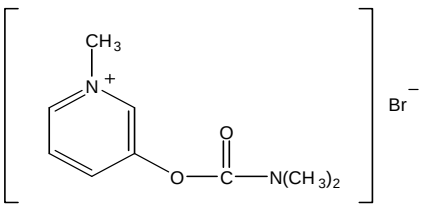
Flow rate: 1 mL/minute.

System suitability

Selection of column: Proceed with 10 μL of the standard solution according to the above operating conditions: the number of theoretical plates and symmetry factor of the peak of pyrazinamide are not less than 2500 and not more than 1.3, respectively. To 1 mL of hydrochloric acid, add the standard solution to make 5 mL, allow to stand in the boiling water-bath for 5 minutes, cool, and proceed with 20 μL of this solution under the above operation conditions: pyrazinoic acid and Pyrazinamide are eluted in this order with the resolution between their peaks being not less than 6.0.

Containers and Storage ***Containers***—Well-closed containers.

Pyridostigmine Bromide



$C_9H_{13}BrN_2O_2$: 261.12

(1-Methylpyridin-1-ium-3-yl) *N,N*-dimethylcarbamate bromide [*101-26-8*]

Pyridostigmine Bromide, when dried, contains not less than 98.5 % and not more than 101.0 % of pyridostigmine bromide ($C_9H_{13}BrN_2O_2$).

Description Pyridostigmine Bromide is as a white, crystalline powder, is odorless or has a slightly characteristic odor.
Pyridostigmine Bromide is very soluble in water, freely soluble in ethanol (95) or acetic acid (100), and practically insoluble in ether.

pH—The pH of a solution of Pyridostiginine Bromide (1 in 10) is between 4.0 and 6.0.
Pyridostigmine Bromide is deliquescent.

Identificatlon (1) Dissolve 20 mg of Pyridostigmine Bromide in 10 mL of water, add 5 mL of Reinecke salt TS: a pale red precipitate is produced.

(2) Take 0.1 g of Pyridostigmine Bromide, add 0.6 mL of sodium hydroxide TS: the unpleasant odor of dimethylamine is perceptible.

(3) Determine the absorption spectra of solutions of Pyridostigmine Bromide and Pyridostigmine Bromide RS, respectively, in 0.1 mol/L hydrochloric acid TS (1 in 30000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) A solution of Pyridostigmine Bromide (1 in 50) responds to the Qualitative Tests for bromide.

Melting Point 153 ~ 157 °C

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Pyridostigmine Bromide in 10 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Pyridostigmine Bromide according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Pyridostigmine Bromide according to Method 1 and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 0.10 g of Pyridostigmine Bromide in 10 mL of ethanol (95) and use this solution as the test solution. Pipet 2 mL of the test solution and add ethanol (95) to make exactly 10 mL. Pipet 1 mL of this solution, add ethanol (95) to make exactly 25 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, chloroform and ammonium chloride TS (5 : 4 : 1) to a distance of about 12 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot from the test solution is not more intense than the spot from the standard solution.

Loss on Drying Not more than 2.0 % (1 g, in vacuum, P_2O_5, 100 °C, 5 hours).

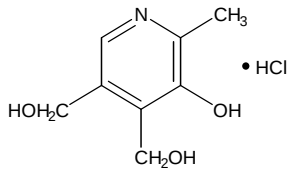
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.3 g of Pyridostigmine Bromide, previously dried, dissolve in 10 mL of acetic acid (100), add 40 mL of acetic anhydride and titrate with 0.1 mol/ L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 26.112 mg of $C_9H_{13}BrN_2O_2$

Containers and Storage ***Containers***—Hermetic containers.

Pyridoxine Hydrochloride



Vitamin B_6 Hydrochloride $C_8H_{11}NO_3 \cdot HCl$: 205.64

4,5-Bis(hydroxymethyl)-2-methylpyridin-3-ol hydrochloride [*58-56-0*]

Pyridoxine Hydrochloride, when dried, contains not less than 98.0 % and not more than 101.0 % of pyridoxine hydrochloride ($C_8H_{11}NO_3 \cdot HCl$).

Description Pyridoxine Hydrochloride is a white to pale yellow, crystalline powder.
Pyridoxine Hydrochloride is freely soluble in water, slightly soluble in ethanol (99.5) and practically insoluble in acetic acid (100) or in acetic anhydride.
Pyridoxine Hydrochloride is gradually affected by light.

Melting point—About 206 °C (with decomposition).

Identification (1) Determine the absorption spectra of solutions of Pyridoxine Hydrochloride and Pyridoxine Hydrochloride RS, respectively, in 0.1 mol/L hydrochloric acid TS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry : both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Pyridoxine Hydrochloride and Pyridoxine Hydrochloride RS, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) An aqueous solution of Pyridoxine Hydrochloride (1 in 10) responds to the Qualitative Tests for chloride.

pH The pH of a solution prepared by dissolving 1.0 g of Pyridoxine Hydrochloride in 50 mL of water is between 2.5 and 3.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Pyridoxine Hydrochloride in 20 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Pyridoxine Hydrochloride according to Method 1 and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

(3) ***Related substances***—Dissolve 1.0 g of Pyridoxine Hydrochloride in 10 mL of water, and use this solution as the test solution. Pipet 2.5 mL of the test solution, and add water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 2 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography, and air-dry the plate. Develop the plate with a mixture of acetone, tetrahydrofuran, *n*-hexane and ammonia solution (28) (65 : 13 : 13 : 9) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of sodium carbonate decahydrate in diluted ethanol (99.5) (3 in 10) (1 in 20) on the plate. After air-drying, spray evenly a solution of 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine in ethanol (99.5) (1 in 1000) on the plate, and air-dry: any spot other than the principal spot from the test solution is not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.3 % (1 g, in vacuum, silica gel, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.2 g of Pyridoxine Hydrochloride, previously dried, add 5 mL of acetic acid (100) and 5 mL of acetic anhydride, dissolve by gentle boiling, cool, add 30 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 20.564 mg of $C_8H_{11}NO_3 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Pyridoxine Hydrochloride Injection

Vitamin B_6 Hydrochloride Injection

Pyridoxine Hydrochloride Injection is an aqueous solution for injection. Pyridoxine Hydrochloride Injection contains not less than 95.0 % and not more than 105.0 % of the labeled amount of pyridoxine hydrochloride ($C_8H_{11}NO_3 \cdot HCl$: 205.64).

Method of Preparation Prepare as directed under Injections, with Pyridoxine Hydrochloride.

Description Pyridoxine Hydrochloride Injection is a colorless or pale yellow, clear liquid.
Pyridoxine Hydrochloride Injection is gradually affected by light.

pH—3.0 ~ 6.0.

Identification (1) Take a volume of Pyridoxine Hydrochloride Injection, equivalent to 50 mg of Pyridoxine Hydrochloride according to the labeled amount, add 0.1 mol/L hydrochloric acid TS to make 100 mL. Pipet 2 mL of this solution and add 0.1 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 288 nm and 292 nm.

(2) To a volume of Pyridoxine Hydrochloride Injection, equivalent to 10 mg of pyridoxine hydrochloride according to the labeled amount, add water to make 10 mL, and use this solution as the test solution. Separately, dissolve 10 mg of Pyridoxine Hydrochloride RS in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 2 μL each of the test solution and standard solution on a plate of silica gel for thin layer chromatography, and air-dry plate. Develop the plate with a mixture of acetone, tetrahydrofuran, *n*-hexane and ammonia solution (28) (65 : 13 : 13 : 9) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of sodium carbonate decahydrate in diluted ethanol (99.5) (3 in 10) (1 in 20) on the plate. After air-drying, spray evenly a solution of 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine in ethanol (99.5) (1 in 1000) on the plate: the spots obtained from the test solution and the standard solution are blue in color and have the same R_f value.

Sterility Test It meets the requirement

Bacterial Endotoxins Less than 3.0 EU/mg of pyridoxine hydrochloride

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay Pipet a volume of Pyridoxine Hydrochloride Injection, equivalent to about 40 mg of pyridoxine hydrochloride ($C_8H_{11}NO_3 \cdot HCl$), and add water to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of internal standard solution and dilute with water to make exactly 100 mL and use this solution as the test solution. Separately, weigh accurately about 40 mg of Pyridoxine Hydrochloride RS, previously dried in a desiccator (in vacuum, silica gel) for 4 hours, and dissolve in and water to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of internal standard solution and dilute with water to make exactly 100 mL and use this solution as the standard solution. Perform the test with exactly 20 μL each of the test and the standard solution according the operation conditions in the Assay under Pyridoxine Hydrochloride Tablets.

$$\text{Amount (mg) of pyridoxine hydrochloride } (C_8H_{11}NO_3 \cdot HCl) = \text{Amount (mg) of Pyridoxine Hydrochloride RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—Dissolve 50 mg of Anhydrous Caffeine RS in water to make 100 mL.

Containers and Storage ***Containers***—Hermetic containers.

Storage—Light-resistant.

Pyridoxine Hydrochloride Tablets

Vitamin B_6 Hydrochloride Tablets

Pyridoxine Hydrochloride Tablets contain not less than 95.0 % and not more than 115.0 % of the labeled amount of pyridoxine hydrochloride ($C_8H_{11}NO_3 \cdot HCl$: 205.64).

Method of Preparation Prepare as directed under Tablets, with Pyridoxine Hydrochloride.

Identification Take a quantity of powdered Tablets, equivalent to about 0.1 g of Pyridoxine Hydrochloride according to the labeled amount, add about 5 mL of water. Shake the mixture well, filter into a test tube and add 2 or 3 drops of iron (III) chloride: an orange to deep red color is observed.

Dissolution Test Perform the test with 1 tablet of Pyridoxine Hydrochloride Tablets at 50 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of water as the dissolution solution. Take 20 mL or more of the dissolved solution 45 minutes after starting the test and filter through a membrane filter with a pore size of not more than 0.8 μm. Discard the first 10 mL of the filtrate, use subsequent filtrate as the test solution. Dilute properly with test solution if it is needed. Separately, weigh accurately about 10 mg of Pyridoxine Hydrochloride RS, previously dried, dissolve in ethanol (95) to make exactly 100 mL, then pipet 5 mL of this solution and add water to make exactly 50 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution according to the following operating conditions as directed in the Liquid Chromatography and determine the peak areas of pyridoxine, Q_T and Q_S, from the test solution and the standard solution, respectively: the dissolution rate of Pyridoxine Hydrochloride Tablets in 45 minutes is not less than 75 %.

$$\text{Dissolution rate (\%) with respect to the labeled amount of Pyridoxine Hydrochloride } (C_8H_{11}NO_3 \cdot HCl) = W_S \times \frac{Q_T}{Q_S} \times \frac{90}{C}$$

W_S: Amount (mg) of Pyridoxine Hydrochloride RS

C : Labeled amount (mg) of pyridoxine hydrochloride ($C_8H_{11}NO_3 \cdot HCl$) in 1 tablet

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 3.9 mm in internal diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 to 10 μm in particle diameter).

Mobile phase: Dissolve 0.14 g of sodium 1-hexanesulfonate in 100 mL of a mixture of water, methanol and acetic acid (100) (73 : 27 : 1)

Flow rate: 1 mL/minute

System suitability

System performance: Weigh 20 mg of Pyridoxine Hydrochloride RS and 20 mg of Riboflavin RS, dissolve in the mixture of water, acetonitrile and acetic acid (100) (94 : 5 : 1) at 65 ºC to 70 ºC with occasional shaking, cool and dilute with the mixture of water, acetonitrile and acetic acid (100) (94 : 5 : 1) to 200 mL. When the procedure is run with 10 μL of this solution under the above operating condition, pyridoxine and riboflavin are eluted in this order.

System repeatability: When the test is repeated 5 times with 10 μL each of the upper solution under the above operating conditions, the relative standard deviation of the peak area of pyridoxine is not more than 3.0 %.

Uniformity of Dosage Units It meets the requirement of the Content uniformity test when the test is performed as the following method.

Transfer 1 tablet of Pyridoxine Hydrochloride Tablets,

previously finely powdered, to a 500 mL volumetric flask containing about 300 mL of water, shake for about 30 minutes, and dilute with water to volume. Filter a portion of the mixture, discarding the first 25 mL of the filtrate. Dilute a suitable aliquot of the subsequent filtrate quantitatively and stepwise with diluted hydrochloric acid (1 in 100) so that the concentration of pyridoxine hydrochloride is about 10 μg per mL and use this solutionas test solution. Dissolve about 10 mg of Pyridoxine Hydrochloride RS, accurately weighed, in diluted hydrochloric acid (1 in 100) to make exactly 100 mL. Pipet 5.0 mL of this solution and dilute with diluted hydrochloric acid (1 in 100) to make exactly 50 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at the wavelength of maximum absorbance at about 290 nm, as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of Pyridoxine Hydrochloride } (C_8H_{11}NO_3\cdot HCl) = \frac{T}{D}\times C\times\frac{A_T}{A_S}$$

T: Labeled amount (mg) of pyridoxine hydrochloride in 1 Pyridoxine Hydrochloride Tablet.

D: Dilution factor

C: Concentration (μg/ mL) of Pyridoxine Hydrochloride RS in the standard solution

Assay Weigh accurately and finely powder not less than 20 Pyridoxine Hydrochloride Tablets. Weigh accurately a portion of the powder, equivalent to about 40 mg of pyridoxine hydrochloride ($C_8H_{11}NO_3\cdot HCl$), add water and shake to mix for 15 minutes. Extract with water to make the volume exactly 100 mL. Filter the extract and discard the first 10 mL of the filtrate. Combine the subsequent 5 mL of filtrate with exactly 5 mL of the internal standard solution, dilute with water to make exactly 100 mL, and use this solution as the test solution. Separately, weigh accurately about 40 mg of Pyridoxine Hydrochloride RS, previously dried in a desiccator (in vacuum, silica gel) for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of the solution, add exactly 5 mL of the internal standard solution, dilute with water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Calculate the ratios of the peak areas of pyridoxine hydrochloride, Q_T and Q_S, to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of Pyridoxine Hydrochloride } (C_8H_{11}NO_3\cdot HCl) = \text{Amount (mg) of Pyridoxine Hydrochloride RS}\times\frac{Q_T}{Q_S}$$

Internal standard solution—Weigh accurately about 50 mg of Anhydrous Caffeine RS, and dissilve in with water to make 100mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength : 280 nm)

Column: A stainless steel column, about 4 mm in internal diameter and 15 cm to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Mobile phase: Dissolve about 1.0 g of sodium 1-hexansulfonate in 750 mL of water and add 250 mL of methanol and 10 ml of acetic acid (31).

Flow rate: Adjust the flow rate so that the retention time of pyridoxine is about 5 minutes.

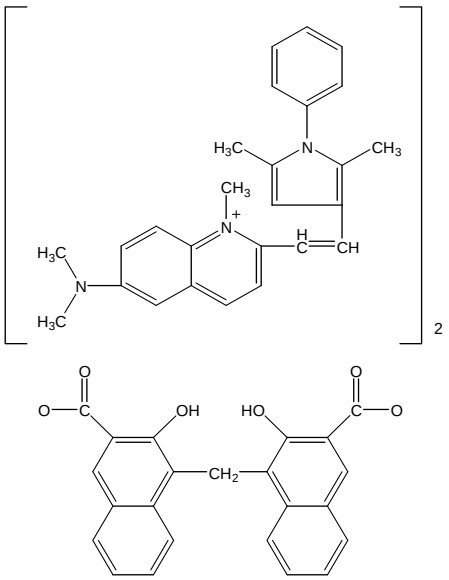
System suitability

Selection of column: Proceed with 20 μL of the standard solution under the above operating conditions, use a column giving elution of pyridoxine hydrochloride and the internal standard in this order with the resolution between their peaks being not less than 3.0.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Pyrvinium Pamoate



$C_{75}H_{70}N_6O_6$: 1151.40

3-Carboxy-1-[(3-carboxy-2-oxidonaphthalen-1-yl)methyl]naphthalen-2-olate [*3546-41-6*]

Pyrvinium Pamoate contains not less than 96.0 % and not more than 104.0 % of pyrvinium pamoate ($C_{75}H_{70}N_6O_6$), calculated on the anhydrous basis.

Description Pyrvinium Pamoate appears as light

orange or blackish orange crystalline powder.
Pyrvinium Pamoate is very soluble in acetic acid (100), slightly soluble in chloroform or in methoxyethanol, very slightly soluble in ethanol (95), and practically insoluble in water or in ether.

Identification (1) Determine the absorption spectrum of the test solution of acetic acid (100) in methanol (1 in 200), prepared as directed in the Assay, as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima at about 358 nm and at about 505 nm and the ratio, A_{505}/A_{358}, is between 1.93 and 2.07.

(2) Determine the infrared spectra of Pyrvinium Pamoate and Pyrvinium Pamoate RS, respectively, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit the same intensities of absorption at the same wavenumbers.

Water Not more than 6.0 % [0.2 g, volumetric titration, direct titration. Use a mixture of methanol and chloroform (1 : 1) instead of methan for water determination].

Residue on Ignition Not more than 0.5 % (1 g).

Assay Weigh accurately about 0.25 g each of Pyrvinium Pamoate and Pyrvinium Pamoate RS, dissolve in 125 mL of acetic acid (100) in a 250 mL volumetric flask and add methanol to make exactly 250 mL, respectively. Pipet 5 mL each of these solutions, add methanol to make exactly 500 mL and use these solutions as the test solution and the standard solution, respectively. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 505 nm as directed under Ultraviolet-visible Spectrophotometry, using methanol as the blank.

$$\text{Amount (mg) of pyrvinium pamoate } (C_{75}H_{70}N_6O_6) = \text{Amount (mg) of Pyrvinium Pamoate RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Tight containers.
Storage—Light-resistant.

Pyrvinium Pamoate Syrup

Pyrvinium Pamoate Syrup contains, in each 100 mL, an amount of Pyrvinium Pamoate equivalent to not less than 0.90 w/v % and not more than 1.10 w/v % of pyrvinium ($C_{26}H_{28}N_3^+$: 382.52).

Method of Preparation Prepare as directed under Syrups, with Pyrvinium Pamoate.

Description Pyrvinium Pamoate Syrup appears as dark red, opaque suspension.

Identification Determine the absorption spectra, between 300 nm and 600 nm, of the test solution and the standard solution obtained in the Assay, respectively, as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit maxima and minima at the same wavelengths.

pH 6.0 ~ 8.0.

Uniformity of Dosage Units (divided) It meets the requirement.

Assay Transfer 50 mg of Pyrvinium Pamoate Syrup (about 5 mL), freshly mixed and free from air bubbles, to a 250 mL volumetric flask. Complete the transfer by rinsing the pipet with 10 mL of methanol, add 100 mL of acetic acid (100), mix to dissolve the Pyrvinium Pamoate, add methanol to make exactly 250 mL and mix. Pipet 3.0 mL of this solution in a 100 mL volumetric flask, dilute with methanol to make exactly 100 mL and use this solution as the test solution. Separately, dissolve accurately weighed a portion of Pyrvinium Pamoate RS (determined formerly water contents as directed under water determination assay) in acetic acid (100), using 4 mL for each 3 mg taken and dilute with methanol to obtain a standard solution having a known concentration of about 9 μg per mL. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 505 nm as directed under Ultraviolet-visible Spectrophotometry, using methanol as the blank.

$$\text{Amount (w/v \%) of pyrvinium } (C_{26}H_{28}N_3^+) = 0.1677 \times C \times \frac{A_T}{A_S} \times 0.6644$$

C: Concentration (μg/mL) of Pyrvinium Pamoate RS in the standard solution

Containers and Storage ***Containers***—Tight containers.
Storage—Light-resistant.

Pyrvinium Pamoate Tablets

Pyrvinium Pamoate Tablets contain not less than 92.0 % and not more than 108.0 % of the labeled amount of pyrvinium ($C_{26}H_{28}N_3^+$: 382.52).

Method of Preparation Prepare as directed under Tablets, with Pyrvinium Pamoate.

Identification Proceed as directed in the Identification under Pyrvinium Pamoate Syrup.

Disintegration Test It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Perform the test without exposure to daylight, using light-resistant vessels. Take a number of Pyrvinium Pamoate Tablets, equivalent to about 0.5 g of pyrvinium ($C_{26}H_{28}N_3^+$), place in a volumetric flask and add 25 mL of water and 25 mL of acetone. Completely disintegrate the tablets by heating in a water-bath for 10 minutes with frequent mixing. To the hot mixture, add 250 mL of acetic acid (100), heat for additional 5 minutes and add methanol to make exactly 500 mL at room temperature. Centrifuge a portion of the mixture until a clear supernatant solution is obtained. Pipet 3 mL of the clear supernatant solution, add methanol to make exactly 500 mL and use this solution as the test solution. Dissolve accurately weighed a portion of Pyrvinium Pamoate RS (determined formerly water contents as directed under water determination assay) in acetic acid (100), using 1 mL for each 3 mg taken and dilute with methanol to obtain a standard solution having a known concentration of about 9 μg per mL. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 505 nm as directed under Ultraviolet-visible Spectrophotometry, using methanol as the blank.

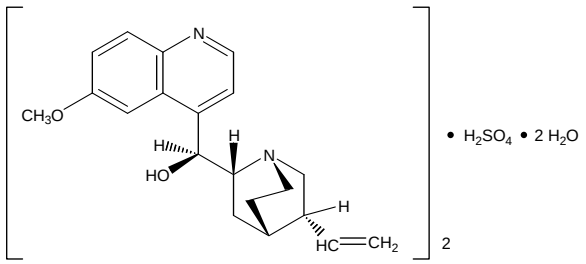
$$\text{Amount (w/w \%) of pyrvinium } (C_{26}H_{28}N_3^+) = 83.3 \times C \times \frac{A_T}{A_S} \times 0.6644$$

C: Concentration (μg/mL) of the standard solution.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Quinidine Sulfate Hydrate



Quinidine Sulfate

$(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O$: 782. 94

(*S*)-[(2*R*,5*R*)-5-Ethenyl-1-azabicyclo[2.2.2]octan-2-yl]-(6-methoxyquinolin-4-yl) methanol; sulfuric acid; dihydrate [*6591-63-5*]

Quinidine Sulfate Hydrate, when dried, contains not less than 98.5 % and not more than 101.0 % of quinidine sulfate [$(C_{20}H_{24}N_2O_2)_2 \cdot 2H_2SO_4$: 746.91]

Description Quinidine Sulfate Hydrate appears as white crystals, is odorless and has a very bitter taste.
Quinidine Sulfate Hydrate is freely soluble in ethanol (95) or in boiling water, sparingly soluble in water and practically insoluble in ether.
Quinidine Sulfate Hydrate, previously dried, is freely soluble in chloroform.
Quinidine Sulfate Hydrate gradually darkens by light.

Specific optical rotation—$[\alpha]_D^{20}$: +275 ~ +287° (after drying, 0.5 g. 0.1 mol/L hydrochloric acid VS, 25 mL, 100 mm).

Identification (1) Dissolve 10 mg of Quinidine Sulfate Hydrate in 10 mL of water and 2 to 3 drops of dilute sulfuric acid: a blue fluorescence is produced.

(2) Take 5 mL of a solution of Quinidine Sulfate Hydrate (1 in 1000), add 1 to 2 drops of bromine TS, then add 1 mL of ammonia TS: a green color develops.

(3) Take 5 mL of a solution of Quinidine Sulfate Hydrate (1 in 100), add 1 mL of silver nitrate TS, stir with a glass rod and allow to stand for a short interval: a white precipitate is produced and it dissolves on drop-wise addition of nitric acid.

(4) Dissolve 0.4 g of Quinidine Sulfate Hydrate in 20 mL of water and 1 mL of dilute hydrochloric acid: the solution responds to the Qualitative Tests for sulfate.

pH Dissolve 1.0 g of Quinidine Sulfate Hydrate in 100 mL of freshly boiled and cooled water: the pH of this solution is between 6.0 and 7.0.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Quinidine Sulfate according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Chloroform-ethanol-insoluble substances***—Warm 2.0 g of Quinidine Sulfate Hydrate with 15 mL of a mixture of chloroform and ethanol (99.5) (2 : 1) at about 50 ºC for 10 minutes. After cooling, filter through a tared glass filter by gentle suction. Wash the residue with five 10 mL volumes of a mixture of chloroform and ethanol (99.5) (2 : 1) and dry at 105 ºC for 1 hour: the weight of the residue is not more than 2.0 mg.

(3) ***Related substances***—Dissolve 20 mg of Quinidine Sulfate Hydrate in the mobile phase to make exactly 100 mL and use this solution as the test solution. Separately, dissolve 25 mg of Cinchonine RS in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform the test with 50 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine the peak areas of each solution by the automatic integration method and calculate their amount by the area percentage method: the amount of dihydroquinine sulfate are not more than 15.0 % and

each amount of quinidine sulfate and dihydroquinine is not more than 1.0 %. The total area of the peaks other than the principal peak and the above peaks is not larger than the peak area of cinchonine from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: A column, about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Mobile phase: A mixture of water, acetonitrile, methanesulfonic acid TS and a solution of diethylamine (1 in 10) (43 : 5 : 1 : 1).

Flow rate: Adjust the flow rate so that the retention time of quinidine is about 10 minutes.

System suitability

Detection sensitivity: Adjust the detection sensitivity so that the peak height of cinchonine obtained from 50 μL of the standard solution is between 5 mm and 10 mm.

System performance: Dissolve 10 mg each of Quinidine Sulfate Hydrate and quinine sulfate in 5 mL of methanol and add the mobile phase to make exactly 50 mL. When the procedure is run with 50 μL of this solution under the above operating conditions, quinidine, quinine, dihydroquinidine and dihydroquinine are eluted in this order with the resolution between quinidine and quinine and that between quinine and dihydroquinidine being not less than 1.2, respectively.

Time span of measurement: About twice as long as the retention time of quinidine after the solvent peak.

(4) ***Readily carbonizable substances***—Take 0.2 g of Quinidine Sulfate Hydrate and perform the test: the solution has no more color than Color Matching Fluid M.

Loss on Drying Not more than 5.0 % (1 g, 130 ºC, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

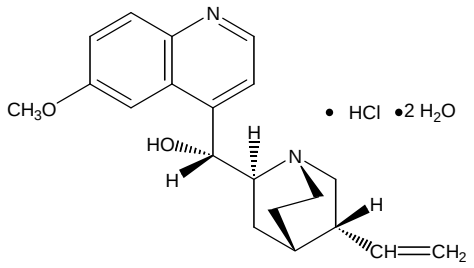
Assay Weigh accurately about 0.5 g of Quinidine Sulfate Hydrate, previously dried, dissolve in 20 mL of acetic acid (100) and add 80 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 3 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 24.897 mg of $(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Quinine Hydrochloride Hydrate



$C_{20}H_{24}N_2O_2.HCl \cdot 2H_2O$: 396.91

(*R*)-[(2*S*,4*S*,5*R*)-5-Ethenyl-1-azabicyclo[2.2.2]octan-2-yl]-(6-methoxyquinolin-4-yl) methanol dihydrate hydrochloride [*6119-47-7*]

Quinine Hydrochloride Hydrate, when dried, contains not less than 98.5 % and not more than 101.0 % of quinine hydrochloride ($C_{20}H_{24}N_2O_2 \cdot HCl$).

Description Quinine Hydrochloride Hydrate appears as white crystals, is odorless and has a very bitter taste.
Quinine Hydrochloride Hydrate gradually changes to brown by light.
Quinine Hydrochloride Hydrate is very soluble in ethanol (99.5), freely soluble in ethanol (95), in acetic acid (100), or in acetic anhydride, sparingly soluble in water, and practically insoluble in ether.
Quinine Hydrochloride Hydrate, previously dried, is freely soluble in chloroform.

Identification (1) A solution of Quinine Hydrochloride Hydrate (1 in 50) shows no fluorescence. To 1 mL of the solution, add 100 mL of water and 1 drop of dilute sulfuric acid: a blue fluorescence is observed.

(2) Take 5 mL of a solution of Quinine Hydrochloride Hydrate (1 in 1000) and add 1 to 2 drops of bromine TS and 1 mL of ammonia TS: a green color develops.

(3) Take 5 mL of a solution of Quinine Hydrochloride Hydrate (1 in 50) and add 1 mL of dilute nitric acid and 1 mL of silver nitrate TS: a white precipitate is produced. Collect the precipitate and add an excess of ammonia TS: it dissolves.

Specific Optical Rotation $[\alpha]_D^{20}$: -245 ~ -255° (0.5 g after drying, 0.1 mol/L hydrochloric acid, 25 mL, 100 mm).

pH Dissolve 1.0 g of Quinine Hydrochloride Hydrate in 100 mL of freshly boiled and cooled water: the pH of this solution is between 6.0 and 7.0.

Purity (1) ***Sulfate***—Perform the test with 1.0 g of Quinine Hydrochloride Hydrate. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS

(not more than 0.048 %).

(2) ***Barium***—Dissolve 0.5 g of Quinine hydrochloride Hydrate in 10 mL of water by warming and add 1 mL of dilute sulfuric acid: no turbidity is produced.

(3) ***Chloroform-ethanol-insoluble substances***—Warm 2.0 g of Quinine Hydrochloride Hydrate with 15 mL of a mixture of chloroform and ethanol (99.5) (2 : 1) at 50 °C for 10 minutes. After cooling, filter through a tared glass filter (G4) by gentle suction. Wash the residue with five times with 10 mL volumes of a mixture of chloroform and ethanol (99.5) (2 : 1), dry at 105 °C for 1 hour and weigh: the weight of the residue so obtained is not more than 2.0 mg.

(4) ***Related substances***—Dissolve 20 mg of Quinine Hydrochloride Hydrate in the mobile phase to make exactly 100 mL and use this solution as the test solution. Separately, dissolve 25 mg of cinchonidine in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform the test with 50 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine the peak areas of each solution by the automatic integration method and calculate the amount of dihydroquinine hydrochloride by the area percentage method: it is not more than 10.0 %. The total area of the peaks other than the principal peak and the above peaks is not larger than the peak area of cinchonidine from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Mobile phase: A mixture of water, acetonitrile, methanesulfonic acid TS and a solution of diethylamine (1 in 10) (43 : 5 : 1 : 1).

Flow rate: Adjust the flow rate so that the retention time of quinine is about 10 minutes.

System suitability

Detection sensitivity: Adjust the detection sensitivity so that the peak height of cinchonidine from 50 μL of the standard solution is between 5 mm and 10 mm.

System performance: Dissolve 10 mg each of Quinine Hydrochloride Hydrate and quinidine sulfate in 5 mL of methanol and add the mobile phase to make exactly 50 mL. When the procedure is run with 50 μL of this solution under the above operating conditions, quinidine, quinine , dihydroquinidine and dihydroquinine are eluted in this order with the resolution between quinidine and quinine and that between quinine and dihydroquinidine being not less than 1.2.

Time span of measurement: About 2 times as long as the retention time of quinine after the solvent peak.

(5) ***Readily carbonizable substances***—Perform the test with 0.25 g of Quinine Hydrochloride Hydrate. The solution has no more color than Color Matching Fluid M.

Loss on Drying Not more than 10.0 % (1 g, 105 °C, 5 hours).

Residue on Ignition Not more than 0.1 % (1 g).

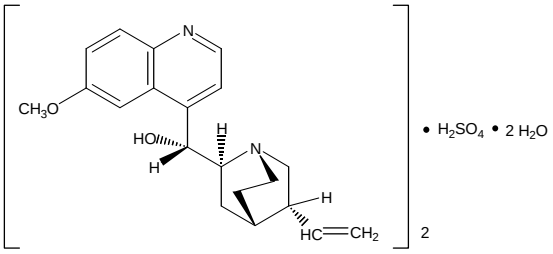
Assay Weigh accurately about 0.4 g of Quinine Hydrochloride Hydrate, previously dried, dissolve in 100 mL of a mixture of acetic acid anhydride and acetic acid (100) (7 : 3) by warming, cool and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 18.044 mg of $C_{20}H_{24}N_2O_2 \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Quinine Sulfate Hydrate



Quinine Sulfate $(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O$: 782.94

(*R*)-[(2*S*,5*R*)-5-Ethenyl-1-azabicyclo[2.2.2]octan-2-yl]-(6-methoxyquinolin-4-yl) methanol; sulfuric acid; dihydrate [*6119-70-6*]

Quinine Sulfate Hydrate contains not less than 98.5 % and not more than 101.0 % of quinine sulfate $[(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4$:746.91], calculated on the dried basis.

Description Quinine Sulfate Hydrate appears as white crystals or crystalline powder, is odorless and has a very bitter taste.

Quinine Sulfate Hydrate is freely soluble in acetic acid (100), soluble in hot ethanol, sparingly soluble in hot water, slightly soluble in water, in ethanol (95), in ethanol (99.5) or in chloroform and practically insoluble in ether.

Quinine Sulfate Hydrate gradually changes to brown by light.

Identification (1) Determine the absorption spectra of solutions of Quinine Sulfate Hydrate and Quinine Sulfate Hydrate RS (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Quinine Sulfate Hydrate and Quinine Sulfate Hydrate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Add 20 mL of water and 1 mL of dilute hydrochloric acid to 0.4 g of Quinine Sulfate Hydrate: this solution responds to the Qualitative Tests for sulfate.

Specific Optical Rotation $[\alpha]_D^{20}$: -235 ~ -245° (after drying, 0.75 g, 0.1 mol/L hydrochioric acid VS, 25 mL, 100 mm).

pH Shake 2.0 g of Quinine Sulfate Hydrate in 20 mL of freshly boiled and cooled water and filter: the pH of this filtrate is between 5.5 and 7.0.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Quinine Sulfate Hydrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Chloroform·ethanol-insoluble substances***—Warm 2.0 g of Quinine Sulfate Hydrate with 15 mL of a mixture of chloroform and ethanol (99.5) (2 : 1) at 50 ºC for 10 minutes. After cooling, filter through a tared glass filter (G4) by gentle suction. Wash the residue with five 10 mL volumes of a mixture of chloroform and ethanol (99.5) (2 : 1), dry at 105 ºC for 1 hour and weigh: the weight of the residue is not more than 2.0 mg.

(3) ***Related substances***—Dissolve 20 mg of Quinine Sulfate Hydrate in the mobile phase to make exactly 100 mL and use this solution as the test solution. Separately, dissolve 25 mg of cinchonidine in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform the test with 50 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine the peak areas of each solution by the automatic integration method and calculate the amount of dihydroquinine sulfate by the area percentage method: it is not more than 5 %. The total area of the peaks other than the principal peak and the above peak is not larger than the peak area of cinchonidine from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: A column, about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Mobile phase: A mixture of water, acetonitrile, methanesulfonic acid TS and a solution of diethylamine (1 in 10) (43 : 5 : 1 : 1).

Flow rate: Adjust the flow rate so that the retention time of quinine is about 10 minutes.

System suitability

Detection sensitivity: Adjust the detection sensitivity so that the peak height of cinchonidine obtained from 50 μL of the standard solution is between 5 mm and 10 mm.

System performance: Dissolve 10 mg each of Quinine Sulfate Hydrate and quinidine sulfate in 5 mL of methanol and add the mobile phase to make exactly 50 mL. When the procedure is run with 50 μL of this solution under the above operating conditions, quinidine, quinine, dihydroquinidine and dihydroquinine are eluted in this order with the resolution between quinidine and quinine and that between quinine and dihydroquinidine being not less than 1.2.

Time span of measurement: About twice as long as the retention time of quinindine after the solvent peak.

Loss on Drying Not more than 5.0 % (1 g, 105 ºC, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

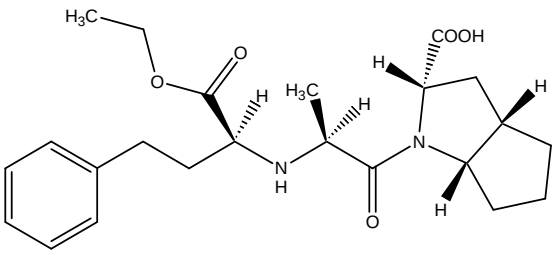
Assay Weigh accurately about 0.5 g of Quinine Sulfate Hydrate, dissolve in 20 mL of acetic acid (100), add 80 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 2 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 24.90 mg of $(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Ramipril



$C_{23}H_{32}N_2O_5$: 416.51

(2*S*,3a*S*,6a*S*)-1-[(2*S*)-2-[[(2*S*)-1-Ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]-3,3a,4,5,6,6a-hexahydro-2*H*-cyclopenta[b]pyrrole-2-carboxylic acid [*87333-19-5*]

Ramipril contains not less than 98.0 % and not more than 101.0 % of ramipil ($C_{23}H_{32}N_2O_5$), calculated on the dried basis.

Description Ramipril is a white, crystalline powder. Ramipril is freely soluble in methanol and sparingly soluble in water.

Identification Determine the infrared spectra of Ramipril and Ramipril RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 105 ~ 112 °C

Specific Optical Rotation $[\alpha]_D^{20}$: +32 ~ +38° (0.25 g, after drying, methanolic hydrochloric acid TS, 25 mL, 100 mm).

Purity (1) ***Palladium***—Weigh accurately about 0.2 g of Ramipril, dissolve in a mixture of water and nitric acid (997:3) to make exactly 100 mL and use this solution as the test solution. Separately, weigh accurately about 50 mg of palladium, add 9 mL of hydrochloric acid to dissolve and add water to make exactly 100 mL. Pipet three suitable portions of this solution, add a mixture of water and nitric acid (997:3) to render the concentration of the resulting solutions having 0.02, 0.03 and 0.05 μg per mL, respectively, and use these solutions as the standard solutions. Separately, weigh accurately about 0.15 g of magnesium nitrate, add a mixture of water and nitric acid (997:3) to make exactly 100 mL and use this solution as the blank solution. Perform the test with 20 μL of the test solution, 20 μL each of the standard solutions and 10 μL of the blank solution as directed in the calibration curve method under the Atomic Absorption Spectrophotometry according to the following conditions and calculate the content of palladium in the test solution not more than 0.002 %.

$$\text{Content (\%) of palladium} = 0.1 \times \frac{\left[\begin{array}{l}\text{Concentration } (\mu\text{g/mL}) \text{ of} \\ \text{palladium in the test solution}\end{array}\right]}{\text{Concentration (mg/mL) of Ramipril in the test solution}}$$

Gas: Dissolved acetylene or hydrogen – Air
Lamp: A palladium hollow cathode lamp
Wavelength: 247.6 nm

(2) ***Related substances***—Weigh accurately about 25 mg of Ramipril, dissolve in the mobile phase A to make exactly 25 mL and use this solution as the test solution. Separately, weigh accurately about 25 mg of Ramipril RS, dissolve in the mobile phase B to make exactly 50 mL. To 1.0 mL of this solution, add the mobile phase B to make exactly 100 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, determine the areas of peaks in the test solution and the standard solution, and calculate the content of related substances according to the following equation: not more than 0.5 % each for ramipril related substance I, ramipril related substance II, ramipril related substance III or ramipril related substance IV, not more than 0.1 % for any other related substance and not more than 1.0 % for total related substances.

$$\text{Content (\%) of related substance} = 100 \times F \times \frac{C_S}{C_T} \times \frac{A_T}{A_S}$$

F: Relative response factor for related substance (2.4 for ramipril related substance III and 1.0 for any other related substance)

C_S*:* Concentration (mg/mL) of ramipril in the test solution

C_T*:* Concentration (mg/mL) of ramipril in the standard solution

A_T*:* Area of each peak obtained from the test solution

A_S*:* Area of ramipril peak obtained from the standard solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column about 4.0 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 65 °C.

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: Dissolve 2.0 g of sodium perchlorate in a mixture of 800 mL of water and 0.5 mL of triethylamine, adjust the pH of the solution to 3.6 ± 0.1 with phosphoric acid, add 200 mL of acetonitrile and mix.

Mobile phase B: Dissolve 2.0 g of sodium perchlorate in a mixture of 300 mL of water and 0.5 mL of triethylamine, adjust the pH of the solution to 2.6 ± 0.1 with phosphoric acid, add 700 mL of acetonitrile and mix.

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)

0-6	90	10
6-7	90→75	10→25
7-20	75→65	25→35
20-30	65→25	35→75
30-40	25	75
40-45	25→90	75→10
45-55	90	10

Flow rate: 1.0 mL/min.

System suitability

System performance: Proceed with 10 μL of the standard solution under the above operating conditions and adjust the conditions so that the retention time for ramipil is between 16 and 19 minutes at the 75:25 stage. Separately, weigh 5.0 mg each of Ramipril RS, ramipril related substance I RS, ramipril related substance II RS, ramipril related substance III RS and ramipril related substance IV RS, and dissolve in 10 mL of the mobile phase B. When the procedure is run with 10 μL of this solution under the above operating conditions, the relative retention times are about 0.8, 1.0, 1.3 and 1.5 for ramipril related substance I, ramipril related substance II, ramipril related substance III and ramipril related substance IV, respectively, with the resolution between the ramipril peak and the ramipril related substance I peak being not less than 3.0. In addition, when the procedure is run with 10 μL of the test solution under the above operating conditions, the retention time for ramipril is between 16 and 19 minutes with the symmetry factor being between 0.8 and 2.0.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the areas of ramipril peak is not more than 5.0 %.

Loss on Drying Not more than 0.2 % (1 g, in vacuum, 60 °C, 6 hours).

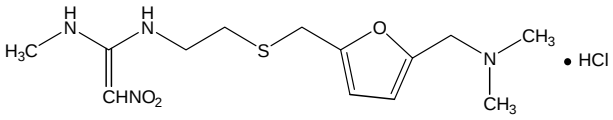
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.3 g of Ramipril, dissolve in 25 mL of methanol, add 25 mL of water and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 41.651 mg of $C_{23}H_{32}N_2O_5$

Containers and Storage ***Containers***—Tight containers.

Ranitidine Hydrochloride



$C_{13}H_{22}N_4O_3S \cdot HCl$: 350.86

(*E*)-*N*-(2-(((5-((Dimethylamino)methyl)furan-2-yl)methyl)thio)ethyl)-*N*-methyl-2-nitroethene-1,1-diamine hydrochloride [*66357-59-3*]

Ranitidine Hydrochloride contains not less than 97.5 % and not more than 102.0 % of ranitidine hydrochloride ($C_{13}H_{22}N_4O_3S \cdot HCl$), calculated on the dried basis.

Description Ranitidine Hydrochloride is a white to pale yellow crystalline powder and is odorless.

Ranitidine Hydrochloride is very soluble in water, freely soluble in methanol and slightly soluble in ethanol (99.5).

Ranitidine Hydrochloride is hygroscopic.

Ranitidine Hydrochloride is gradually colored by light.

Melting point—About 140 °C (with decomposition).

Identification (1) Determine the absorption spectra of the solutions of Ranitidine Hydrochloride and Ranitidine Hydrochloride RS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Ranitidine Hydrochloride and Ranitidine Hydrochloride RS, previously dried, as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensity of absorption at the same wavenumbers.

(3) A solution of Ranitidine Hydrochloride (1 in 50) responds to the Qualitative Tests for chloride.

pH Dissolve 1.0 g of Ranitidine Hydrochloride in 100 mL of water: the pH of this solution is between 4.5 and 6.0.

Purity (1) ***Clarity and color of solution***—A solution of Ranitidine Hydrochloride is clear and pale yellow to light yellow.

(2) ***Heavy metals***—Proceed with 2.0 g of Ranitidine Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Proceed with 1.0 g of Ranitidine Hydrochloride according to Method 4 and perform the test (not more than 2 ppm).

(4) ***Related substances***— Dissolve about 0.2 g of Ranitidine Hydrochloride, accurately weighed, in 10 mL of methanol, and use this solution as the test solution. Dissolve about 20 mg of Ranitidine Hydrochloride RS, accurately weighed, in 10 mL of methanol. Pipet 1.0 mL of this solution, dilute with methanol to

make exactly 10mL, and use this solution as the standard solution (1). Dilute the standard solution (1) with methanol to obtain the standard solutions (2), (3) and (4), having known concentrations of 0.1 mg/mL, 60 μg/mL and 10 μg/mL, respectively. Separately, dissolve a suitable quantity of Ranitidine related compound I RS in methanol to obtain a solution containing 1.3 mg per mL, and use this solution as the resolution solution. And dissolve a suitable quantity of Ranitidine related compound II RS in methanol to obtain a solution having a known concentration of about 1 mg/mL, and use this solution as the identification solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution, the standard solutions (1), (2), (3), (4) and the identification solution, on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Separately, spot 10 μL of the test solution on the same plate, and on top of this spot, spot 10 μL of the resolution solution. Then, develop the plate with a mixture containing ethyl acetate, 2-propanol, ammonia solution (28) and water (25 : 15 : 5 : 1) to a distance of about 15 cm, and air-dry the plate. Expose the plate to iodine vapor: any spots from the test solution corresponding to the R_f values of the principal spot obtained from the identification solution are not greater in size or intensity than the principal spots obtained from the standard solutions (2) (not more than 0.5 %). The spots other than the principal spot are not greater in size or intensity, as compared to the principal spot obtained from the standard solution (3) (not more than 0.3 %). The sum of spots other than the principal spot, obtained from the test solution, is not more than 1.0 % of total. The spot is observed in the chromatogram of standard solution (4), and there is complete resolution between the principal spots in the chromatogram of the combined test solution and resolution solution.

Loss on Drying Not more than 0.75 % (1 g, 60 °C, in vacuum, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Transfer about 0.1 g of Ranitidine Hydrochloride, accurately weighed, to a 100 mL volumetric flask. Dissolve in and dilute with a mobile phase to volume. Transfer 1.0 mL of this solution to a 10 mL volumetric flask, dilute with the mobile phase to the volume, and use this as the test solution. Dissolve an accurately weighed quantity of Ranitidine Hydrochloride RS in the mobile phase to obtain a solution containing 0.1 mg per mL, and use this as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine the peak areas, A_T and A_S, of ranitidine hydrochloride for the test solution and the standard solution, respectively.

Amount (mg) of ranitidine hydrochloride

$$(C_{13}H_{22}N_4O_3S\cdot HCl) = C \times \frac{A_T}{A_S} \times 1000$$

C: Concentration of ranitidine hydrochloride in the standard solution (mg/mL).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 322 nm).

Column: A stainless steel column, 4.6 mm in internal diameter and 20 cm to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm to 10 μm in particle diameter).

Mobile phase: A degassesd and filtered mixture of methanol and 0.1 mol/L aqueous ammonium acetate (85 : 15).

Flow rate: 2 mL/minute.

System suitability

System performance: Dissolve an accurately weighed quantity of Ranitidine Hydrochloride RS and Ranitidine related compound III RS in a mobile phase to obtain a solution containing 0.1 mg and 0.01 mg per mL, respectively. When the procedure is run with 10 μL each of these solutions under the above operating conditions, the resolution between their peaks is not less than 1.5, and the symmetry factor of ranitidine hydrochloride peak is not more than 2.0. The column efficiency determined from ranitidine hydrochloride peak is not less than 700 theoretical plates.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of ranitidine hydrochloride is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Ranitidine Hydrochloride Tablets

Ranitidine Hydrochloride Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of ranitidine ($C_{13}H_{22}N_4O_3S$: 314.40).

Method of Preparation Prepare as directed under Tablets, with Ranitidine Hydrochloride.

Identification (1) The principal spots from the test solution and the standard solution as directed in the Related substances under the Purity show the same R_f value.

(2) The retention time of the principal peak in the chromatogram of the test solution corresponds to that of the standard solution as obtained in the Assay.

(3) Take a portion of powdered Ranitidine Hydrochloride Tablets, equivalent to 0.1 g Ranitidine Hydro-

chloride according to the labeled amount, add 2 mL of water, shake well, and filter. The filtrate responds to the Qualitative Tests for chloride.

Purity ***Related substances*—**Shake an appropriate number of Ranitidine Hydrochloride Tablets in a suitable volume of methanol until the tablets have disintegrated completely, and filter. Pipet a suitable volume of the filtrate, dilute with methanol to obtain a solution containing 20 mg of ranitidine per mL (equivalent to 22.4 mg of Ranitidine Hydrochloride per mL), and use this as the test solution. Dissolve Ranitidine Hydrochloride RS in methanol to obtain a solution having a known concentration of 0.22 mg/mL, and use this solution as the standard stock solution. Dilute portions of the standard stock solution with methanol to obtain the standard solutions (1), (2), (3) and (4), having known concentrations of about 110 μg/mL, 66 μg/mL, 22 μg/mL and 11 μg/mL, respectively. Dissolve a suitable quantity of Ranitidine Hydrochloride related compound I RS, accurately weighed, in methanol to obtain a solution having a known concentration of about 1.27 mg/mL, and use this solution as the resolution solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution, the standard stock solution, the standard solutions (1), (2), (3) and (4) on a plate of silica gel for thin-layer chromatography. Then, develop the plate with a mixture containing ethyl acetate, 2-propanol, ammonia solution (28), and water (25 : 15 : 5 : 1) to a distance of about 15 cm, and air-dry the plate. Expose the plate to iodine vapor: the spots other than the principal spot from the test solution are not more intense than the principal spot obtained from the standard solution (1). No single secondary spot exhibits intensity greater than that of the standard solution (1) (not more than 0.5 %), and no other secondary spot exhibits intensity greater than that of the standard solution (2) (0.3 %). The sum of the intensities of all secondary spots obtained from the test solution corresponds to not more than 2.0 %. A spot is observed in the chromatogram of the standard solution (4), and there is complete resolution between the principal spots in the chromatogram of the combined test solution and resolution solution.

Dissolution Test Perform the test with 1 tablet of Ranitidine Hydrochloride Tablets at 50 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of water as the dissolution solution. Take 20 mL of the dissolved solution after 45 minutes from start of the test, and filter through a membrane filter with pore size of not more than 0.8 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the test solution. Separately, take about 0.22 g of Ranitidine Hydrochloride RS, accurately weighed, and add water to make exactly 100 mL. Pipet 1.0 mL of this solution, transfer this to a 100 mL volumetric flask, dilute with water to volume. Pipet 1.0 mL of this solution, transfer this to a 100 mL volumetric flask, dilute with water to volume, and use this as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution at the maximum absorption wavelength about 314 nm as directed under Ultraviolet-visible Spectrophotometry, using the dissolution solution as the blank.

The dissolution rate of Ranitidine Hydrochloride Tablets in 45 minutes is not less than 80 %.

Uniformity of Dosage Units It meets the requirement when the content uniformity test is performed according to the procedure as directed in the Assay under Ranitidine Hydrochloride Tablets for the uniformity of the content.

Assay Weigh accurately and powder not less than 20 Ranitidine Hydrochloride Tablets. Weigh accurately a portion of the powder, equivalent to 10 Ranitidine Hydrochloride Tablets, add in excess of 250 mL of the mobile phase and mix until the mixture is completely dispersed. Filter the mixture and dilute a suitable portion of the filtrate with the mobile phase to render the concentration identical to the standard solution. Use this solution as the test solution. Separately, weigh accurately Ranitidine Hydrochloride RS, dissolve in the mobile phase to render the concentration of the solution to be 0.12 mg in 1 mL. Use this solution as the standard solution. Perform the test with the operating condition directed in the Assay under Ranitidine Hydrochloride and the Liquid Chromatography. Measure A_T for the peak area of ranitidine in the test solution and A_S for the peak area of ranitidine in the standard solution.

$$\text{Amount (mg) of ranitidine } (C_{13}H_{22}N_4O_3S) = C \times \frac{A_T}{A_S} \times \frac{314.40}{350.87} \times \frac{L}{D}$$

C: Concentration of the standard solution (mg/mL)

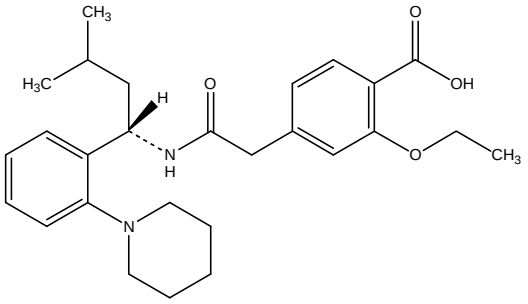
L: Labeled amount of ranitidine in one tablet (mg)

D: Concentration of ranitidine in the test solution according to the labeled amount of ranitidine in one tablet (mg/mL)

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Repaglinide



$C_{27}H_{36}N_2O_4$: 452.59

(*S*)-2-Ethoxy-4-[2-(3-methyl-1-[2-(piperidin-1-yl)phenyl]butylamino)-2-oxoethyl]benzoic acid [*135062-02-1*]

Repaglinide contains not less than 98.0 % and not more than 101.0 % of repaglinide ($C_{27}H_{36}N_2O_4$), calculated on the dried basis.

Description Repaglinide is a white solid.
Repaglinide is soluble in methanol.

Melting point—132 ~ 136 °C

Identification (1) Determine the absorption spectra of solutions of Repaglinide and Repaglinide RS in methanol (1 in 40000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Repaglinide and Repaglinide RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +6.3 ~ +7.3° (0.5 g, methanol, 10 mL, 100 mm).

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Repaglinide according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Weigh accurately about 0.1 g of Repaglinide, dissolve in methanol to make exactly 10 mL and use this solution as the test solution. To 1.0 mL of the test solution, add methanol to make exactly 10 mL and use this solution as the standard solution. Perform the test with 3 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the areas of peaks other than the principal peaks by the automatic integration method: not more than 0.1 % for each related substance and not more than 0.5 % for total related substances. Multiply the equation by a response factor of 2 for the repaglinide related substance I {(*S*)-3-methyl-1-[2-(1-piperidinyl)phenyl]butylamine, *N*-acetyl-L-glutamate salt}.

$$\text{Content (\%) of each related substance} = \frac{A_i}{A_S}$$

A_i: Peak area of each related substance obtained from the test solution

A_S: Peak area of repaglinide obtained from the standard solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 12.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45 °C.

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: Dissolve 3 g of potassium dihydrogen phosphate in 1000 mL of water and adjust the pH of the solution to 7.0 by the addition of 1 mol/L sodium hydroxide TS.

Mobile phase B: Methanol

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0	50	50
0-2	50→30	50→70
2-8	30	70
8-12	30→5	70→95
12-15	5	95

Flow rate: 1.0 mL/min.

System suitability

System performance: Weigh 0.1 g of Repaglinide RS, 1 mg each of repaglinide related substance I RS, repaglinide related substance II (3-ethoxy-4-ethoxycarbonylphenylacetate) RS and repaglinide related substance III {(*S*)-2-ethoxy-4-[2-[[2-phenyl-1-[2-(1-piperidinyl)phenyl]ethyl]amino]-2-oxoethyl] benzoate} RS, dissolve in methanol to make 10 mL and use this solution as the test solution for system performance. When the procedure is run with 20 μL of the test solution for system performance under the above operating conditions, the relative retention times are 0.3, 0.6 and 1.6 for the related substance II, III and I, respectively.

System repeatability: When the test is repeated 6 times with 3 μL each of the standard solution under the above operating conditions, the relative standard deviation of the areas of repaglinide peak is not more than 10 %.

Loss on Drying Not more than 0.5 % (30 mg, 105 °C, constant mass).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 25 mg each of Repaglinide and Repaglinide RS, dissolve in methanol to make exactly 50 mL each and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the area of repaglinide peak in test solution, A_T, and in the standard solution, A_S.

$$\text{Amount (mg) of repaglinide } (C_{27}H_{36}N_2O_4) = \text{Amount (mg) of Repaglinide RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 12.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45 °C.

Mobile phase: A mixture of methanol and phosphate buffer (800:200)

Flow rate: 1.0 mL/min

System suitability

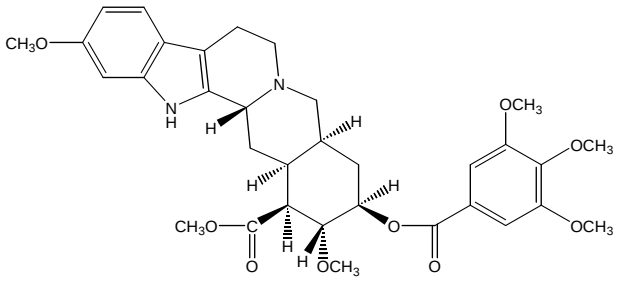
System performance: Dissolve 25 mg of Repaglinide RS and 2 mg of repaglinide related substance II RS in methanol to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the relative retention times are about 1.0 and about 0.4 for repaglinide peak and the related substance II peak, respectively.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the areas of repaglinide peak is not more than 2.0 %.

Phosphate buffer—To the solution of potassium dihydrogen phosphate (1 in 1000), add phosphoric acid to adjust the pH of the solution to 2.5.

Containers and Storage ***Containers***—Tight containers.

Reserpine



$C_{33}H_{40}N_2O_9$: 608.68

Methyl(1*R*,15*S*,17*R*,18*R*,19*S*,20*S*)-6,18-di-methoxy-17-(3,4,5-trimethoxybenzoyl)oxy-1,3,11,12,14,15,16,17,18,19,20,21-dodecahydro-yohimban-19-carboxylate [*50-55-5*]

Reserpine, when dried, contains not less than 96.0 % and not more than 101.0 % of reserpine ($C_{33}H_{40}N_2O_9$).

Description Reserpine appears as white to pale yellow crystals or crystalline powder and is odorless.
Reserpine is freely soluble in acetic acid (100) or in chloroform, slightly soluble in acetonitrile, very slightly soluble in ethanol (95) and practically insoluble in water or in ether.
Reserpine is affected by light.

Identification (1) Take 1 mg of Reserpine, add 1 mL of vanillin-hydrochloric acid TS and warm: a vivid red-purple color is observed.

(2) Determine the absorption spectra of solutions of Reserpine and Reserpine RS in acetonitrile (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Reserpine and Reserpine RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers

Specific Optical Rotation $[\alpha]_D^{20}$: -114 ~ -127° (after drying, 0.25 g, chloroform, 25 mL, 100 mm).

Purity ***Related substances***—Perform the test without exposure to daylight, using light-resistant vessels. Dissolve 50 mg of Reserpine in 50 mL of acetonitrile and use this solution as the test solution. Pipet 3.0 mL of the test solution, add acetonitrile to make exactly 100 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine each peak area from test solution and the standard solution by the automatic integration

method: the total area of peaks other than reserpine peak from the test solution is not larger than the peak area of reserpine from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 268 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: A mixture of 0.05 mol/L monobasic potassium phosphate, pH 3.0 and acetonitrile (13 : 7).

Flow rate: Adjust the flow rate so that the retention time of reserpine is about 20 minutes.

System suitability

Detection sensitivity: Adjust the detection sensitivity so that the peak height of reserpine from 10 μL of the standard solution is about 20 mm.

System performance: Dissolve 10 mg of Reserpine and 4 mg of butyl paraoxybenzoate in 100 mL of acetonitrile. To 5 mL of this solution, add acetonitrile to make exactly 50 mL. When the procedure is run with 20 L of this solution according to above operating conditions, reserpine and butyl parahydroxybenzoate are eluted in this order with the resolution between their peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of reserpine is not more than 2.0 %.

Time span of measurement: About twice as long as the retention time of reserpine.

Loss on Drying Not more than 0.5 % (0.2 g, in vacuum, 60 °C, 3 hours).

Residue on Ignition Not more than 0.2 % (0.2 g).

Assay Perform the test without exposure to daylight, using light-resistant vessels. Weigh accurately about 10 mg each of Reserpine and Reserpine RS, previously dried and dissolve each in acetonitrile to make exactly 100 mL. Pipet 5.0 mL each of these solutions, add exactly 10 mL of the internal standard solution, 5 mL of acetonitrile and water to make exactly 50 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and calculate the ratios, Q_T and Q_S, of the peak area of reserpine to that of the internal standard for the test solution and the standard solution, respectively,

$$\text{Amount (mg) of reserpine } (C_{33}H_{40}N_2O_9) = \text{Amount (mg) of Reserpine RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of butyl paraoxybenzoate in acetonitrile (1 in 50000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 268 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: A mixture of 0.05 mol/L monobasic potassium phosphate, pH 3.0 and acetonitrile (11 : 9).

Flow rate: Adjust the flow rate so that the retention time of reserpine is about 10 minutes.

System suitability

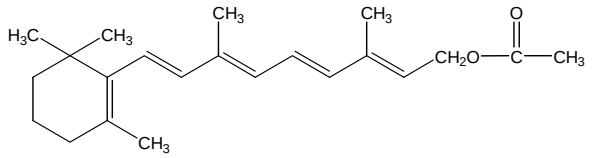
System performance: When the procedure is run with 20 μL of the standard solution according to above operating conditions, reserpine and the internal standard are eluted in this order with the resolution between their peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of peak area of reserpine to that of the internal standard is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Retinol Acetate



Vitamin A Acetate $C_{22}H_{32}O_2$: 328.49

(2*E*,4*E*,6*E*,8*E*)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenylacetate [*127-47-9*]

Retinol Acetate is synthetic retinol acetate or synthetic retinol acetate diluted with fixed oil. Retinol Acetate contains not less than 2500000 vitamin A units per gram. A suitable antioxidant may be added. Retinol Acetate contains not less than 95.0 % and not more than 105.0 % of the labeled units.

Description Retinol Acetate appears as pale yellow to yellow-red crystals or an ointment-like substance

and has a faint, characteristic odor, but has no rancid odor.
When powdered, Retinol Acetate is very soluble in chloroform or in ether, freely soluble in petroleum ether, soluble in ethanol (95) or in 2-propanol and practically insoluble in water.
Retinol Acetate is affected by air and by light

Identification Dissolve separately Retinol Acetate and Retinol Acetate RS, equivalent to 15000 Units each, in 5 mL each of petroleum ether, and use these solutions as the test solution and standard solution, respectively. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of cyclohexane and ether (12:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly antimony (III) chloride TS: the principal spot obtained from the test solution is the same in color tone and R_f value with the blue spot from the standard solution.

Purity (1) ***Acid value***—Take exactly 5.0 g of Retinol Acetate, and perform the test : not more than 2.0.

(2) ***Peroxide***—Weigh accurately about 5 g of Retinol Acetate, transfer in a 250 mL glass-stoppered conical flask, add 50 mL of a mixture of acetic acid (100) and isooctane (3:2), and gently mix to dissolve. Replace the air of the inside gradually with about 600 mL of Nitrogen, then add 0.1 mL of saturated potassium iodide TS under a current of Nitrogen. Immediately stopper tightly, and mix with a swirling motion for 1 minute. Add 30 mL of water, stopper tightly, and shake vigorously for 5 to 10 seconds. Titrate this solution with 0.01 mol/L sodium thiosulfate VS until the blue color of the solution disappears after addition of 0.5 mL of starch TS near the end point where the solution is a pale yellow color. Calculate the amount of peroxide by the following formula: not more than 10 meq/kg.

$$\text{Amount (meq/kg) of peroxide} = (V/W) \times 10$$

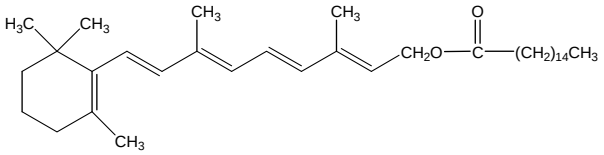
V: Volume (mL) of 0.01 mol/L sodium thiosulfate VS consumed
W: Amount (g) of the test specimen

Assay Proceed as directed for Method 1 under the Vitamin A Assay.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, almost well-filled or under nitrogen atmosphere, and in a cold place.

Retinol Palmitate



Vitamin A Palmitate
Retinol Palmitinic Acid $C_{36}H_{60}O_2$: 524.86

(2*E*,4*E*,6*E*,8*E*)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenylhexadecanoate [*79-81-2*]

Retinol Palmitate is a synthetic retinol palmitate or a synthetic retinol palmitate diluted with fixed oil and contains not less than 1500000 vitamin A units in each gram. Retinol Palmitate may contain a suitable antioxidant. Retinol Palmitate contains not less than 95.0 % and not more than 105.0 % of the labeled units.

Description Retinol Palmitate is a pale yellow to yellow-red, ointment-like or an oily substance. Retinol Palmitate has a faint, characteristic odor, but has no rancid odor.
Retinol Palmitate is very soluble in petroleum ether, slightly soluble in ethanol (95) and practically insoluble in water.
Retinol Palmitate is affected by air and by light.

Identification Dissolve separately Retinol Palmitate and Retinol Palmitate RS, equivalent to 15000 Units each, in 5 mL each of petroleum ether, and use these solutions as the test solution and the standard solution, respectively. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of cyclohexane and ether (12:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly antimony (III) chloride TS: the principal spot obtained from the test solution is the same in color tone and R_f value with the blue spot from the standard solution.

Purity (1) ***Acid value***—Take exactly 5.0 g of Retinol Acetate, and perform the test : not more than 2.0.

(2) ***Peroxide***—Weigh accurately about 5 g of Retinol Palmitate, transfer in a 250 mL glass-stoppered conical flask, add 50 mL of a mixture of acetic acid (100) and isooctane (3:2), and gently mix to dissolve. Replace the air of the inside gradually with about 600 mL of Nitrogen, then add 0.1 mL of saturated potassium iodide TS under a current of Nitrogen. Immediately stopper tightly, and mix with a swirling motion for 1 minute. Add 30 mL of water, stopper tightly, and shake vigorously for 5 to 10 seconds. Titrate this solution with 0.01 mol/L sodium thiosulfate VS until the blue

color of the solution disappears after addition of 0.5 mL of starch TS near the end point where the solution is a pale yellow color . Calculate the amount of peroxide by the following formula: not more than 10 meq/kg.

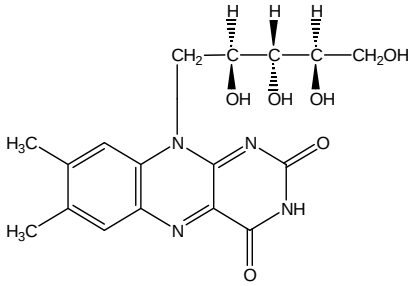
Amount (meq/kg) of peroxide = $(V/W) \times 10$ mol/L

V: Volume (mL) of 0.01 mol/L sodium thiosulfate VS consumed
W: Amount (g) of the test specimen

Assay Proceed as directed in Method 1 under the Vitamin A Assay.

Containers and Storage ***Containers***—Tight containers.
Storage—Light-resistant, almost well-filled or under nitrogen atmosphere, and in a cold place.

Riboflavin



Vitamin B_2 $C_{17}H_{20}N_4O_6$: 376.36

7,8-Dimethyl-10-[(2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl]benzo[g]pteridine-2,4-dione
[*83-88-5*]

Riboflavin, when dried, contains not less than 98.0 % and not more than 101.0 % of riboflavin ($C_{17}H_{20}N_4O_6$).

Description Riboflavin appears as yellow to orange crystals and has a slight odor.
Riboflavin is very slightly soluble in water, practically insoluble in ethanol (95), in acetic acid (100) or in ether.
Riboflavin dissolves in sodium hydroxide TS.
A saturated solution of Riboflavin is neutral.
Riboflavin is decomposed by light.
Melting point—About 290 °C (with decomposition).

Identification (1) The solution of Riboflavin (1 in 100000) is pale yellow-green in color and has an intense yellow-green fluorescence. The color and fluorescence of the solution disappear upon the addition of 20 mg of sodium hydrosulfite to 5 mL of the solution and reappear on shaking the mixture in air. This fluorescence disappears upon the addition of dilute hydrochloric acid or sodium hydroxide TS.

(2) Take 10 mL of a solution of Riboflavin (1 in 100000), place in a glass-stoppered test tube, add 1 mL of sodium hydroxide TS and after illumination with a fluorescence lamp of 10 to 30 watts at 20 cm distance for 30 minutes between 20 °C and 40 °C, acidify with 0.5 mL of acetic acid (31) and shake with 5 mL of chloroform: the chloroform layer shows a yellow-green fluorescence.
(3) Determine the absorption spectra of solutions of Riboflavin and Riboflavin RS in phosphate buffer solution, pH 7.0 (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Specific Optical Rotation $[\alpha]_D^{20}$: -128 ~ -142°.
Weigh accurately about 0.1 g of Riboflavin, previously dried, dissolve in 4.0 mL of dilute sodium hydroxide TS. Add 10 mL of freshly boiled and cooled water and 4.0 mL of aldehyde-free alcohol while shaking, add freshly boiled and cooled water to make exactly 20 mL and determine the optical rotation in a 100 mm cell within 30 minutes after preparing the solution.

Purity ***Lumiflavin***—Shake 25 mg of Riboflavin with 10 mL of ethanol-free chloroform for 5 minutes and filter: the filtrate has no more color than the following control solution.

Control solution—Take 2.0 mL of 0.0167 mol/L potassium bichromate VS, add water to make exactly 1000 mL.

Loss on Drying Not more than 1.5 % (0.5 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.2 % (1 g).

Assay Perform the test without exposure to daylight, using light-resistant vessels. Weigh accurately about 15 mg of Riboflavin, previously dried, dissolve in 800 mL of diluted acetic acid (100) (1 in 400) by warming, cool, add water to make exactly 1000 mL and use this solution as the test solution. Weigh accurately about 15 mg of Riboflavin RS, previously dried at 105 °C for 2 hours, dissolve in 800 mL of diluted acetic acid (100) (1 in 400) by warming, cool, add water to make exactly 1000 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry, using water as the blank and determine the absorbances, A_T and A_S, at 445 nm for the test solution and the standard solution, respectively,. Add 20 mg of sodium hydrosulfite to 5 mL of each solution, shake until decolorized and immediately measure the absorbances, A'_T and A'_S, of the test solution and the standard solution, respectively.

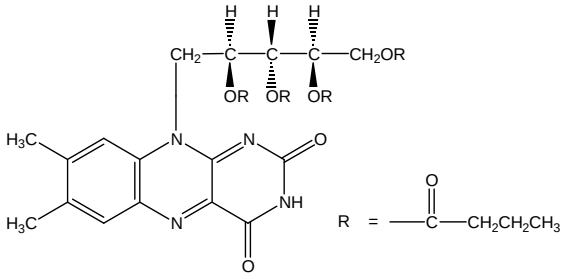
Amount (mg) of riboflavin ($C_{17}H_{20}N_4O_6$)

$$= \text{Amount (mg) of Riboflavin RS} \times \frac{A_T - A'_T}{A_S - A'_S}$$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Riboflavin Butyrate



$C_{33}H_{44}N_4O_{10}$: 656.72

[(2*R*,3*S*,4*S*)-2,3,4-Tri(butanoyloxy)-5-(7,8-dimethyl-2,4-dioxobenzo[g]pteridin-10-yl)pentyl]butanoate
[*752-56-7*]

Riboflavin Butyrate, when dried, contains not less than 98.5 % and not more than 101.0 % of riboflavin butyrate ($C_{33}H_{44}N_4O_{10}$).

Description Riboflavin Butyrate appears as orange crystals, crystalline powder and has a slight, characteristic odor and a slightly bitter taste.
Riboflavin Butyrate is freely soluble in methanol, in ethanol (95) or in chloroform, slightly soluble in ether and practically insoluble in water.
Riboflavin Butyrate is decomposed by light.

Identification (1) A solution of Riboflavin Butyrate in ethanol (95) (1 in 100000) shows a pale yellow color with strong yellowish green fluorescence. To this solution, add dilute hydrochloric acid or sodium hydroxide TS: the fluorescence disappears.

(2) Dissolve 10 mg of Riboflavin Butyrate in 5 mL of ethanol (95), add 2 mL of a mixture of a solution of hydroxylamine hydrochloride (3 in 20) and a solution of sodium hydroxide (3 in 20) (1 : 1) and shake well. To this solution, add 0.8 mL of hydrochloric acid and 0.5 mL of iron (III) chloride TS and add 8 mL of ethanol (95): a deep red-brown color is observed.

(3) Determine the absorption spectra of solutions of Riboflavin Butyrate and Riboflavin Butyrate RS in ethanol (4 in 250000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting Point 146 ~ 150 °C.

Purity (1) ***Chloride***—Dissolve 2.0 g of Riboflavin Butyrate in 10 mL of methanol and add 24 mL of dilute nitric acid and water to make exactly 100 mL. After shaking well, allow to stand for 10 minutes, filter, discard the first 10 mL of the filtrate and use the subsequent filtrate as the test solution. To 25 mL of the test solution, add water to make exactly 50 mL, then add 1 mL of silver nitrate TS and allow to stand for 5 minutes: the turbidity of the solution is not thicker than that of the following control solution.

Control solution—Take 25 mL of the test solution, add 1 mL of silver nitrate TS, allow to stand for 10 minutes and filter. Wash the precipitate with four 5 mL volumes of water and combine the washings with the filtrate. To this solution, add 0.30 mL of 0.01 mol/L hydrochloric acid VS and water to make exactly 50 mL, add 1 mL of water and mix (not more than 0.021 %).

(2) ***Heavy metals***—Proceed with 2.0 g of Riboflavin Butyrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Free acid***—Take 1.0 g of Riboflavin Butyrate, add 50 mL of freshly boiled and cooled water, shake and filter. To 25 mL of the filtrate, add 0.50 mL of 0.01 mol/L sodium hydroxide VS and 2 drops of phenolphthalein TS: the solutions shows a red color.

(4) ***Related substances***—Dissolve 0.10 g of Riboflavin Butyrate in 10 mL of chloroform and use this solution as the test solution. Pipet 1.0 mL of the test solution and add chloroform to make exactly 50 mL. Pipet 5 mL of this solution, add chloroform to make exactly 20 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 L each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and 2-propanol (9 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot form the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, silica gel, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Perform the test without exposure to daylight, using light-resistant vessels. Weigh accurately about 40 mg of Riboflavin Butyrate, previously dried, and dissolve in ethanol (95) to make exactly 500 mL. Pipet 10.0 mL of this solution, add ethanol (95) to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately about 50 mg of Riboflavin RS, previously dried at 105 °C for 2 hours, dissolve in 150 mL of diluted acetic acid (100) (2 in 75) by warming and after cooling, add water to make exactly

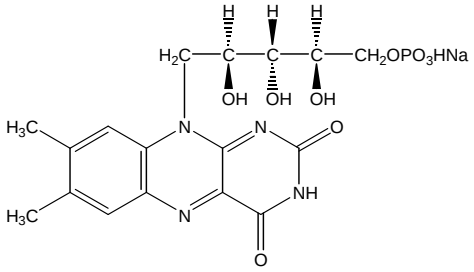
500 mL. Pipet 5.0 mL of this solution, add ethanol (95) to make exactly 50 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 445 nm as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of riboflavin butyrate } (C_{33}H_{44}N_4O_{10}) = \text{Amount (mg) of Riboflavin RS} \times \frac{A_T}{A_S} \times 1.7449 \times \frac{1}{2}$$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Riboflavin Sodium Phosphate



Riboflavin Phosphate
Vitamin B_2 Phosphate Ester $C_{17}H_{20}N_4NaO_9P$: 478.33

Sodium[(2*S*,3*R*,4*R*)-5-(7,8-dimethyl-2,4-dioxobenzo[g]pteridin-10-yl)-2,3,4-trihydroxypentyl] hydrogen phosphate [*130-40-5*]

Riboflavin Sodium Phosphate contains not less than 92.0 % and not more than 101.0 % of riboflavin sodium phosphate ($C_{17}H_{20}N_4NaO_9P$), calculated on the anhydrous basis.

Description Riboflavin Sodium Phosphate appears as yellow to orange, crystalline powder, is odorless and has a slightly bitter taste.
Riboflavin Sodium phosphate is soluble in water and practically insoluble in ethanol (95), chloroform or in ether.
Riboflavin Sodium Phosphate is decomposed by light.
Riboflavin Sodium Phosphate is very hygroscopic.

Identification (1) A solution of Riboflavin Sodium Phosphate (1 in 100000) is pale yellow-green in color and has an intense yellow-green fluorescence. The color and fluorescence of the solution disappear upon the addition of 20mg of sodium hydrosulfite to 5 mL of the solution and reappear on shaking the mixture in air. This fluorescence disappears upon the addition of dilute hydrochloric acid or sodium hydroxide TS.

(2) Take 10 mL of a solution of Riboflavin Sodium Phosphate (1 in 100000) placed in a glass-stoppered test tube, add 1 mL of sodium hydroxide TS and after illumination with a fluorescence lamp of 10 to 30 watts at 20 cm distance for 30 minutes between 20 °C and 40 °C, acidify with 0.5 mL of acetic acid (31) and shake with 5 mL of chloroform: the chloroform layer shows a yellow-green fluorescence.

(3) Determine the absorption spectra of solutions of Riboflavin Sodium Phosphate and Riboflavin Sodium Phosphate RS in phosphate buffer solution, pH 7.0 (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Take 50mg of Riboflavin Sodium Phosphate, add 10 mL of nitric acid, evaporate on a water-bath to dryness and ignite. Boil the residue with 10 mL of diluted nitric acid (1 in 50) for 5 minutes, after cooling, neutralize this solution with ammonia TS and filter, if necessary: the solution responds to the Qualitative Tests for sodium salt and phosphate.

Specific Optical Rotation $[\alpha]_D^{20}$: +38 ~ +43° (0.3 g, calculated on the anhydrous basis, 5 mol/L hydrochloric acid TS, 20 mL, 100 mm).

pH Dissolve 0.20 g of Riboflavin Sodium Phosphate in 20 mL of water: the pH of the solution is between 5.0 and 6.5.

Purity (1) ***Clarity and color of solution***—Dissolve 0.20 g of Riboflavin Sodium Phosphate in 10 mL of water: the solution is clear and yellow to orange in color.

(2) ***Heavy metals***—Proceed with 2.0 g of Riboflavin Sodium Phosphate according to Method 2 under Heavy Metals Limit Test and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Lumiflavin***—To 35 mg of Riboflavin Sodium Phosphate, add 10 mL of ethanol-free chloroform and shake for 5 minutes, then filter: the filtrate has no more color than the control solution.

Control solution—Take 3.0 mL of 0.0167 mol/L potassium dichromate VS, add water to make 1000 mL.

(4) ***Free phosphoric acid***—Weigh accurately about 0.4 g of Riboflavin Sodium Phosphate, dissolve in water to make exactly 100 mL and use this solution as the test solution. Measure exactly 5 mL each of the test solution and phosphoric acid standard solution, respectively, transfer volumetric flasks, add 2.5 mL of ammonium molybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS to each of these flasks, mix and add water to make exactly 25 mL. Allow to stand for 30 minutes at 20 ± 1 °C and perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry, using a solution prepared with 5 mL of water in the same manner as a

blank. Determine the absorbances, A_T and A_S, of the subsequent solutions of the test solution and the phosphoric acid standard solution at 740 nm: the free phosphoric acid content is not more than 1.5 %.

$$\text{Amount (\%) of free phosphoric acid } (H_3PO_4) = \frac{A_T}{A_S} \times \frac{1}{W} \times 257.8$$

W: Amount (mg) of Riboflavin Sodium Phosphate calculated on the anhydrous basis.

Water Place 25 mL of a mixture of methanol for Water Determination and ethylene glycol for Water Determination (1 : 1) in a dry flask for titration and titrate with water determination TS to the end point. Weigh accurately about 0.1 g of Riboflavin Sodium Phosphate, place quickly into the flask, add a known excess volume of Karl Fischer TS, mix for 10 minutes and perform the test: the water content is not more than 10.0 %.

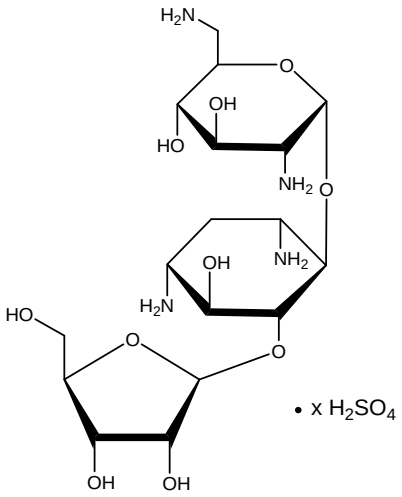
Assay Perform the test without exposure to daylight, using light-resistant vessels. Weigh accurately about 0.1 g of Riboflavin Sodium Phosphate, dissolve in diluted acetic acid (100) (1 in 500) to make exactly 1000 mL. Pipet 10 mL of this solution and add diluted acetic acid (100) (1 in 500) to make exactly 50 mL, use this solution as the test solution. Separately, weigh accurately about 15 mg Riboflavin RS, previously dried at 105 °C for 2 hour, dissolve in 800 mL of diluted acetic acid (1 in 400) by warming, cool add water to make exactly 1000 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry, using water as the blank. Determine the absorbances, A_T and A_S, at 445 nm. Add 20 mg of sodium hydrosulfite to 5 mL of each solution, shake until decolorized and immediately measure the absorbances, A'_T and A'_S, of the solutions.

$$\text{Amount (mg) of riboflavin sodium phosphate } (C_{17}H_{20}N_4NaO_9P) = \text{Amount (mg) of Riboflavin RS} \times \frac{A_T - A'_T}{A_S - A'_S} \times 1.2709 \times 5$$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Ribostamycin Sulfate



$C_{17}H_{34}N_4O_{10} \cdot xH_2SO_4$

5-Amino-2-(aminomethyl)-6-[4,6-diamino-2-[(2*S*,4*R*,5*R*)-3,4-dihydroxy-5-(hydroxy-methyl)oxolan-2-yl]oxy-3-hydroxycyclohexyl]oxyoxane-3,4-diol; sulfuric acid [*53797-35-6*]

Ribostamycin Sulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of *Streptomyces ribosidificus*. Ribostamycin Sulfate contains not less than 680 μg (potency) and not more than 780 μg (potency) per mg of ribostamycin ($C_{17}H_{34}N_4O_{10}$: 454.47), calculated on the dried basis.

Description Ribostamycin Sulfate appears as white to pale yellow powder.
Ribostamycin Sulfate is very soluble in water and practically insoluble in ethanol (95).

Identification (1) Dissolve 20 mg of Ribostamycin Sulfate in 2 mL of phosphate buffer solution (pH 6.0), add 1 mL of ninhydrin TS and heat: a blue-purple color develops.

(2) Dissolve 0.12 g each of Ribostamycin Sulfate and Ribostamycin Sulfate RS in 20 mL of water and use these solutions as the test solution and the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (3 in 40) to a distance of about 10 cm and air-dry the plate. Spray evenly 0.2 % ninhydrin-water saturated 1-butanol TS and heat at 100 °C for 10 minutes: the principal spots obtained from the test solution and the spots obtained from the standard solution show a purple-brown color and the same R_f value.

(3) To 2 mL of a solution of Ribostamycin Sulfate (1 in 5), add 1 drop of barium chloride TS: a white turbidity is produced.

Specific Optical Rotation $[\alpha]_D^{20}$: +42 ~ +49° (0.25 g after drying, water, 25 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g (potency) of Ribostamycin Sulfate in 20 mL of water is between 6.0 and 8.0.

Purity (1) ***Clarity and color of solution*—**Dissolve 1.0 g of Ribostamycin Sulfate in 5 mL of water: the solution is clear and colorless to pale yellow.

(2) ***Heavy metals*—**Proceed with 1.0 g of Ribostamycin Sulfate according to Method 1 and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

(3) ***Arsenic*—**Proceed with 1.0 g of Ribostamycin Sulfate according to Method 1 and perform the test (not more than 2 ppm).

(4) ***Related substances*—**Dissolve 0.12 g of Ribostamycin Sulfate in water to make 20 mL and use this solution as the test solution. Pipet 5 mL of this solution, add water to make exactly 100 mL and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (3 in 40) to a distance of about 10 cm and air-dry the plate. Spray evenly 0.2 % ninhydrin-water saturated 1-butanol TS and heat at 100 °C for 10 minutes: the spot other than the principal spot obtained from the test solution is not more intense than the spot from the standard solution.

Loss on Drying Not more than 5.0 % (0.5 g, not exceeding 0.67 kPa, 60 °C, 3 hours).

Residue on Ignition Not more than 1.0 % (1 g).

Sterility Test It meets the requirement, when Ribostamycin Sulfate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.50 EU/mg (potency) of ribostamycin, when used in a sterile preparation**.**

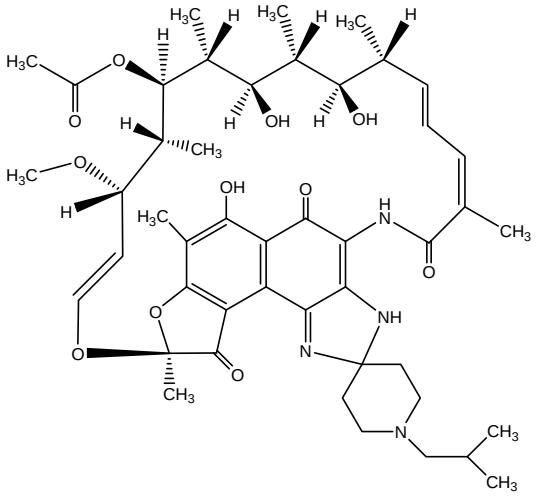
Assay ***The Cylinder-plate method*** (1) Agar media for seed and base layer- Use the culture medium in I 2 1) (1) ① under Microbial Assay for Antibiotics.

(2) Test organism- *Bacillus subtilis* ATCC 6633

(3) Weigh accurately an amount of Ribostamycin Sulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 50 mL. Pipet a suitable amount of this solution, dilute with 0.1 mol/L phosphate buffer (pH 8.0) to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency) and use these solutions as the high concentration test solution and the low concentration test solution, respectively. Separately, weigh accurately about 20 mg (potency) of Ribostamycin Sulfate RS, previously dried, dissolve in diluted phosphate buffer (pH 6.0) (1 in 2) to make exactly 50 mL and use this solution as the standard stock solution. Keep the standard stock solution at 5 °C to 15 °C and use within 20 days. Pipet a suitable amount of the standard stock solution, dilute with 0.1 mol/L phosphate buffer (pH 8.0) to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency) and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively. Perform the test with these solutions as directed in I 8 under Microbial Assay for Antibiotics.

Containers and Storage ***Containers*—**Tight containers.

Rifabutin



$C_{46}H_{62}N_4O_{11}$: 847.01

(9*S*,12*E*,14*S*,15*R*,16*S*,17*R*,18*R*,19*R*,20*S*,21*S*,22*E*,24*Z*)-6,16,18,20-Tetrahydroxy-1‘-isobutyl-14-methoxy-7,9,15,17,19,21,25-heptamethylspiro[9,4-(epoxypentadeca[1,11,13]trienimino)-2*H*-furo[2‘,3‘:7,8]naphth[1,2-d]imidazole-2,4‘-piperidine]-5,10,26-(3*H*,9*H*)-trione-16-acetate [*72559-06-9*]

Rifabutin contains not less than 950 μg (potency) and not more than 1020 μg (potency) per mg of rifabutin ($C_{46}H_{62}N_4O_{11}$: 847.01), calculated on the anhydrous basis.

Description Rifabutin appears as red-purple powder. Rifabutin is soluble in methanol or in chloroform, sparingly soluble in ethanol (95) and slightly soluble in water.

Identification (1) Determine the infrared spectra of Rifabutin and Rifabutin RS as directed in the potassium bromide disk method under Infrared Spectropho-

tometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) When proceed as directed in the Assay, the retention time of the principal peak from the test solution corresponds to that from the standard solution.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Rifabutin according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Perform the test as directed in the Assay, determine each peak area in the test solution by the automatic integration method and calculate the amount of each related substance by the area percentage method: the amount of each related substance having the relative retention time of about 0.5, 0.6, 0.8 or 1.4 with respect to rifabutin is not more than 1.0 %, the amount of any other related substance is not more than 0.5 % and the total amount of related substances is not more than 3.0 %.

(3) ***Isobutylpiperidone***—Weigh accurately about 0.1 g (potency) of Rifabutin, dissolve in a mixture of chloroform and methanol (1 : 1) so that each mL contains 10 mg (potency) and use this solution as the test solution. Separately, weigh accurately about 0.1 g of Isobutylpiperidone RS and dissolve in a mixture of chloroform and methanol (1 : 1) so that each mL contains 1 mg. Pipet 0.5 mL, 1.0 mL, 2.0 mL, 5.0 mL and 10.0 mL of this solution, dilute each with a mixture of chloroform and methanol (1 : 1) to make 100 mL and use these solutions as the standard solutions (S1, S2, S3, S4, S5). Perform the test with the test solution and the standard solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and standard solutions S1, S2, S3, S4 and S5 on a plate of silica gel with fluorescent indicator for thin-layer chromatography, 0.25 mm in thickness. Develop the plate with a mixture of petroleum ether (60 °C to 80 °C) and acetone (10 : 3) to a distance of about 10 cm and air-dry the plate. Expose the plate to iodine vapor for 5 minutes and spray evenly 5 % starch TS: the bluish purple spot of isobutylpiperidone from the test solution is not more intense than the bluish purple spot obtained from standard solution S4 (not more than 0.5 %).

Water Not more than 2.5 % (0.5 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.3 % (1 g).

Assay Weigh accurately about 50 mg (potency) each of Rifabutin and Rifabutin RS, dissolve each in the mobile phase to make exactly 50 mL, pipet 2 mL each of these solutions, add the mobile phase to make exactly 20 mL and use these solutions as the test solution and the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the peak areas, A_T and A_S, of rifabutin in the test solution and the standard solution.

$$\text{Amount [μg (potency)] of rifabutin } (C_{46}H_{62}N_4O_{11}) = \text{Amount [μg (potency)] of Rifabutin RS} \times \frac{A_T}{A_S} \times \frac{100}{100 - m}$$

m: Water content (%)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.7 mm in internal diameter and about 11 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Adjust the pH of a mixture of 0.1 mol/L potassium dihydrogen phosphate TS and acetonitrile (1:1) to 6.5 with 2 mol/L sodium hydroxide TS.

Flow rate: 1.0 mL/minute

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Rifabutin Capsules

Rifabutin Capsules contain not less than 90.0 % and not more than 120.0 % of the labeled amount of rifabutin ($C_{46}H_{62}N_4O_{11}$: 847.01).

Method of Preparation Prepare as directed under Capsules, with Rifabutin.

Identification (1) Weigh 0.2 g (potency) each of Rifabutin Capsules and Rifabutin RS, dissolve each in 200 mL of methanol, filter, take 2 mL each of the filtrates, add methanol to make 100 mL and use these solutions as the test solution and the standard solution. Determine the absorption spectra of the test solution and the standard solution as direted under Ultraviolet-visible Spectrophotometery and compare the spectrum with the spectrum of a standard solution of Rifabutin RS prepared in the same manner as the test solution: both spectra exhibit maxima at the same wavelengths.

(2) When proceed as directed in the Assay, the retention time of the principal peak from the test solution corresponds to that from the standard solution.

Purity ***Related substances***—Weigh accurately not less than 20 Rifabutin Capsules, transfer an accurately weighed portion of the powder, equivalent to about 25 mg (potency) of rifabutin, dissolve in 5 mL of acetonitrile, add the mobile phase to make 50 mL, filter through a filter with a pore size of 0.5 μm and use the filtrate as the test solution. Perform the test with 10 μL of the test solution as directed under Liquid Chroma-

tography according to the following operating conditions. Determine each peak area of the test solution by the automatic integration method and calculate the amount of each related substance by the area percentage method: the amount of each related substance having the relative retention time of 0.5, 0.6, 0.8 or 1.4 with respect to rifabutin is not more than 1.0 %, the amount of any other related substance is not more than 0.5 % and the total amount of related substances is not more than 4.5 %.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 12.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Adjust the pH of a mixture of acetonitrile and 0.1 mol/L potassium dihydrogen phosphate TS (1:1) to 6.5 with 2 mol/L sodium hydroxide.

Flow rate: About 1.0 mL/minute

System suitability

System performance: Dissolve 10 mg of rifabutin in 2 mL of methanol, add 1 mL of 2 mol/L sodium hydroxide, allow to stand for 4 minutes, add 1 mL of 2 mol/L hydrochloric acid and dilute with the mobile phase to make 50 mL. When the procedure is run with this solution under the above operating conditions, one main peak and two minor peaks appear at the relative retention time of about 0.5, 0.6, 0.8 and 1.0 with respect to the peak area of rifabutin, and the resolution between the peak having the relative retention time of about 0.8 with respect to the peak area of rifabutin and the peak of rifabutin is not less than 1.3. When the procedure is run with the system suitability solution under the above operating conditions, the number of theoretical plates is not less than 2000.

System repeatability: Weigh accurately 25 mg of Rifabutin RS, dissolve in 5 mL of acetonitrile, add acetonitrile to make exactly 50 mL and use this solution as the system suitability solution. When the test is repeated 6 times with this solution under the above operating conditions, the relative standard deviation of the peak area is not more than 2.0 %.

Dissolution Test Perform the test with 1 capsule of Rifabutin Capsules at 100 revolutions per minute according to Method 1, using 900 mL of 0.01 mol/L hydrochloric acid TS as the dissolution solution. Take not less than 20 mL of the dissolved solution after 45 minutes from the start of the test, filter, take V mL of the filtrate, add the dissolution solution to make exactly V' mL so that each mL contains about 13.0 μg (potency) of rifabutin and use this solution as the test solution. Separately, weigh accurately about 13 mg (potency) of Rifabutin RS and dissolve in 100 mL of the dissolution solution. Pipet 10 mL of this solution, add the dissolution solution to make exactly 100 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 280 nm of the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry, using the dissolution solution as the blank. The dissolution rate of Rifabutin Capsules in 45 minutes is not less than 75.0 %.

Dissolution rate (%) with respect to the labeled amount of rifabutin ($C_{46}H_{62}N_4O_{11}$) = Amount [mg (potency)] of

$$\text{Rifabutin RS} \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 90$$

C: Labeled amount [mg (potency)] of rifabutin ($C_{46}H_{62}N_4O_{11}$) in 1 capsule

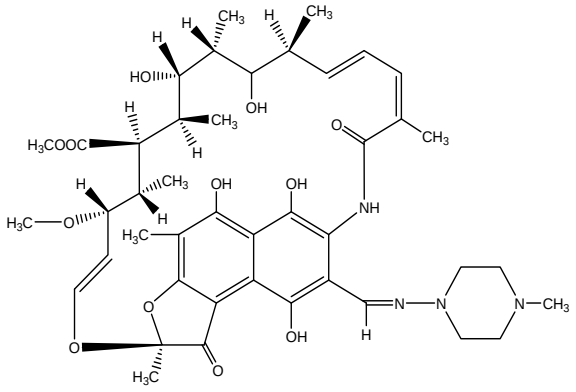
Uniformity of Dosage Units It meets the requirement.

Assay Perform the test as directed in the Assay under Rifabutin. Weigh accurately the contents of not less than 20 Rifabutin Capsules, powder if necessary, weigh accurately a portion of the powder, equivalent to about 50 mg (potency) of rifabutin according to the labeled potency and dissolve in the mobile phase to make exactly 50 mL. To 2 mL of this solution, add the mobile phase to make exactly 20 mL, filter and use the filtrate as the test solution.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Rifampicin



$C_{43}H_{58}N_4O_{12}$: 822.94

(2*S*,12*Z*,14*E*,16*S*,17*S*,18*R*,19*R*,20*R*,21*S*,22*S*,23*R*,24*E*)-5,6,9,17,19-Pentahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-8-(4-methyl-piperazin-1-yliminomethyl)-1,11-dioxo-1,2-dihydro-2,7-(Epoxypentadeca[1,11,13]trien-imino)naphtho[2,1-*b*]furan-21-yl acetate [*13292-46-1*]

Rifampicin is a derivative of a substance having antibacterial activity produced by the growth of *Streptomyces mediterranei*.

Rifampicin contains not less than 970 μg (potency) and not more than 1020 μg (potency) per mg of rifampicin ($C_{43}H_{58}N_4O_{12}$), calculated on the dried basis.

Description Rifampicin appears as orange to red-brown crystals or crystalline powder.
Rifampicin is slightly soluble in water, in methanol, in ethanol (95) or in acetonitrile.

Identification (1) To 5 mL each of the solutions of Rifampicin and Rifampicin RS in methanol (1 in 5000), add 0.05 mol/L phosphate buffer (pH 7.0) to make 100 mL. Determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Rifampicin and Rifampicin RS as directed under the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

pH The pH of a suspension obtained by suspending 0.1 g of Rifampicin in 10 mL of water is between 4.0 and 6.0.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Rifampicin according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Arsenic***—Proceed with 1.0 g of Rifampicin according to Method 3 and perform the test (not more than 2 ppm).

(3) ***Related substances***—Weigh accurately about 0.10 g of Rifampicin, add acetonitrile to make exactly 50 mL and use this solution as the test stock solution. Pipet 5 mL of this solution, add the diluent to make exactly 50 mL and use this solution as the test solution. Separately, pipet 1 mL of the test stock solution, add acetonitrile to make exactly 100 mL, pipet 5 mL of this solution, add the diluent to make exactly 50 mL and use this solution as the standard solution. Perform the test with 50 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine each peak area by the automatic integration method: the peak area of the related substance having the relative retention time of about 0.7 with respect to the peak of rifampicin from the test solution is not larger than 1.5 times the peak area of rifampicin from the standard solution (1.5 %), the area of the peak other than rifampicin and the related substance eluted at the relative retention time of about 0.7 from the test solution is not larger than the peak area of rifampicin from the standard solution (1 %), and total area of these peaks is not larger than 3.5 times the peak area of rifampicin from the standard solution (3.5 %).

Diluent—Weigh accurately 2.1 g of citric acid monohydrate, 13.4 g of dipotassium hydrogen phosphate and 3.1 g of potassium dihydrogen phosphate, dissolve in water to make exactly 1000 mL and mix this solution with acetonitrile in the ratio of 3:1.

Operating conditions

Proceed as directed in the operating conditions in the Assay.

System suitability

Test for required detectability: Pipet 2 mL of the standard solution and add the diluent to make exactly 20 mL. Confirm that the peak area of rifampicin obtained from 50 μL of this solution is equivalent to 7 % to 13 % of that from 50 μL of the standard solution.

System performance: To 5 mL of a solution of rifampicin in acetonitrile (1 in 5000), add 1 mL of a solution of butyl paraoxybenzoate in acetonitrile (1 in 5000), mix and add the diluent to make exactly 50 mL. When the procedure is run with 50 μL of this solution under the above operating conditions, butyl paraoxybenzoate and rifampicin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 50 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rifampicin is not more than 2.0 %.

Time span of measurement: About 3 times as long as the retention time of rifampicin

Loss on Drying Not more than 2.0 % (1.0 g, not exceeding 0.67 kPa, 60 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 40 mg (potency) of Rifampicin and Rifampicin RS and dissolve each in acetonitrile to make exactly 200 mL. Pipet 10 mL each of these solutions, add citric acid-phosphate-acetonitrile TS to make exactly 50 mL and use these solutions as the test solution and the standard solution. Perform the test with 50 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine the peak areas, A_T and A_S, of rifampicin.

$$\text{Amount [μg (potency)] of rifampicin } (C_{43}H_{58}N_4O_{12}) = \text{Amount [μg (potency)] of Rifampicin RS} \times \frac{A_T}{A_S}$$

Diluent—Weigh accurately 2.1 g of citric acid monohydrate, 13.4 g of dipotassium hydrogen phosphate and 3.1 g of potassium dihydrogen phosphate, dissolve in water to make exactly 1000 mL and mix this solution with acetonitrile in the ratio of 3:1.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: A mixture of a solution prepared by dissolving 4.2 g of citric acid monohydrate and 1.4 g of sodium perchlorate in water to make 1000 mL, acetonitrile and pH 3.1 phosphate buffer (11:7:2)

pH 3.1 phosphate buffer—Dissolve 136.1 g of potassium dihydrogen phosphate in 500 mL of water and add 6.3 mL of phosphoric acid and water to make exactly 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of rifampicin is about 8 minutes.

System suitability

System performance: To 5 mL of a solution of rifampicin in acetonitrile (1 in 5000), add 1 mL of a solution of butyl paraoxybenzoate in acetonitrile (1 in 5000) and add citric acid-phosphate-acetontrile TS to make 50 mL. When the procedure is run with 50 μL of this solution under the above operating conditions, butyl paraoxybenzoate and rifampicin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rifampicin is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Rifampicin Capsules

Rifampicin Capsules contain not less than 93.0 % and not more than 105.0 % of the labeled amount of rifampicin ($C_{43}H_{58}N_4O_{12}$: 822.94).

Method of Preparation Prepare as directed under Capsules, with Rifampicin.

Identification (1) Take out the contents of Rifampicin Capsules, mix well and powder, if necessary. Dissolve an amount of the contents, equivalent to about 20 mg (potency) according to the labeled potency, in 100 mL of methanol and filter. To 5 mL of the filtrate, add 0.05 mol/L phosphate buffer (pH 7.0) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 234 and 238 nm, between 252 and 256 nm, between 331 nm and 335 nm and between 472 nm and 476 nm.

(2) Powder the contents of the Rifampicin Capsules, weigh about 0.1 g (potency) accurately of the powder, according to the labeled potency, place it in stoppered-flask, add 10 mL of methanol, mix with vigorously shaking, filter and use the filtrate as the test solution. Separately, dissolve a suitable amount of Rifampicin RS in methanol to make a solution containing 5 mg per mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone or chloroform and ethanol (2 : 1) and air-dry the plate. The red spots obtained from the test solution and the standard solution show the same R_f value.

Purity ***Related substances***—Perform the test quickly after the test solution and the standard solution are prepared. Open the capsules of not less than 20 Rifampicin Capsules, take out the contents, weigh accurately and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg (potency) according to the labeled potency of Rifampicin Capsules, and dissolve in acetonitrile to make exactly 10 mL. Pipet 2 mL of this solution, add a mixture of methanol and acetonitrile (1 : 1) to make exactly 20 mL and use this solution as the test solution. Separately, weigh accurately about 20 mg (potency) of Rifampicin RS and dissolve in acetonitrile to make exactly 10 mL. Pipet 2 mL of this solution and add a mixture of methanol and acetonitrile (1 : 1) to make exactly 20 mL. Pipet 1 mL of this solution, add a mixture of methanol and acetonitrile (1 : 1) to make exactly 50 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine each peak area by the automatic integration method: the amount of the quinone substance and the *N*-oxide substance, having the retention time of about 0.5 and about 1.2 with respect to rifampicin, obtained from the test solution, is not more than 4.0 % and not more than 1.5 %, respectively. The amount of each related substance other than the peaks mentioned above is not more than 1.0 % and the total amount of related substances is 2.0 %. Use the peak areas of the quinone substance and the *N*-oxide substance after multiplying by their response factors, 1.24 and 1.16, respectively.

$$\text{Amount (\%) of related substances} = \frac{W_S}{W_T} \times \frac{A_i}{A_S} \times 2$$

W_S: Amount [mg (potency)] of Rifampicin RS taken

W_T: Amount [mg (potency)] of Rifampicin Capsules

A_S: Peak area of the standard solution

A_i: Peak area of each related substance

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column 4.6 mm in internal diameter and 25 cm in length, packed with octylsilyl silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 2.1 g of sodium perchlorate, 6.5 g of citric acid monohydrate and 2.3 g of potassium dihydrogen phosphate in 1100 mL of water and add 900 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of rifampicin is about 12 minutes.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution and add a mixture of methanol and acetonitrile (1:1) to make exactly 20 mL. Confirm that the peak area of rifampicin obtained from 20 μL of this solution is equivalent to 3.5 % to 6.5 % of that from the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of rifampicin is not less than 2500 with the symmetry factor being not more than 4.0.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rifampicin is not more than 2.0 %.

Time span of measurement: About 2.5 times as long as the retention time of rifampicin

Loss on Drying Not more than 3.0 % (0.1 g, in vacuum, 60 °C, 3 hours).

Dissolution Test Perform the test with 1 capsule of Rifampicin Capsules at 100 revolutions per minute according to Method 1, using 900 mL of 0.1 mol/L hydrochloric acid TS as the dissolution solution. Take the dissolved solution after 45 minutes from the start of the test and filter. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution solution to make exactly V' mL and use this solution as the test solution. Separately, weigh accurately a suitable amount of Rifampicin RS, previously dried, dissolve in the dissolution solution to make the same concentration as the test solution and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 475 nm of the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry, using the dissolution solution as the blank. The dissolution rate of Rifampicin Capsules in 45 minutes is not less than 75 % (Q).

$$\text{Dissolution rate (\%) with respect to the labeled amount of rifampicin } (C_{43}H_{58}N_4O_{12}) = C_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 90000$$

C_S: Concentration [mg (potency)/mL] of the standard solution

C: Labeled amount [mg (potency)] of rifampicin ($C_{43}H_{58}N_4O_{12}$) in 1 capsule

Uniformity of Dosage Units It meets the requirement of the Mass Variation Test.

Assay Open the capsules of not less than 20 Rifampicin Capsules, take out the content, weigh accurately the mass of the content. Weigh accurately an amount of the contents, equivalent to about 75 mg (potency) according to the labeled potency of Rifampicin Capsules, and dissolve in a mixture of methanol and acetonitrile (1:1) to make exactly 50 mL. Pipet 10 mL of this solution and add acetonitrile to make exactly 50 mL. Pipet 5 mL of this solution, add a solution prepared by dissolving 2.1 g of citric acid monohydrate, 27.6 g of disodium hydrogen phosphate dodecahydrate and 3.1 g of potassium dihydrogen phosphate in 1000 mL of a mixture of water and acetonitrile (3 : 1) to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately about 30 mg (potency) of Rifampicin RS, dissolve in 20 mL of a mixture of methanol and acetonitrile (1 : 1) and add acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add a solution prepared by dissolving 2.1 g of citric acid monohydrate, 27.6 g of disodium hydrogen phosphate dodecahydrate and 3.1 g of potassium dihydrogen phosphate in 1000 mL of a mixture of water and acetonitrile (3 : 1) to make exactly 50 mL and use this solution as the standard solution. Perform the test with exactly 50 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and determine the peak areas, A_T and A_S, of rifampicin in each solution.

$$\text{Amount [μg (potency)] of rifampicin } (C_{43}H_{58}N_4O_{12}) = \text{Amount [μg (potency)] of Rifampicin RS} \times \frac{A_T}{A_S} \times \frac{5}{2}$$

Operating conditions

Proceed as directed in the operating conditions in the Assay under Rifampicin.

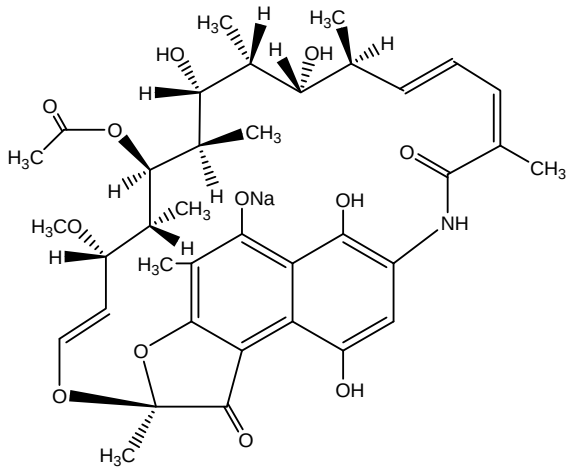
System suitability

System performance: Dissolve 30 mg (potency) of Rifampicin RS in 20 mL of a mixture of methanol and acetonitrile (1 : 1) and add acetonitrile to make 100 mL. To 5 mL of this solution, add 2 mL of a solution of butyl paraoxybenzoate in a mixture of methanol and acetonitile (1 : 1) (1 in 5000) and add a solution prepared by dissolving 2.1 g of citric acid monohydrate, 27.6 g of disodium hydrogen phosphate dodecahydrate and 3.1 g of potassium dihydrogen phosphate in 1000 mL of a mixture of water and acetonitrile (3 : 1) to make 50 mL. When the procedure is run with 50 μL of this solution under the above operating conditions, butyl paraoxybenzoate and rifampicin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rifampicin is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Rifamycin Sodium



$C_{37}H_{46}NNaO_{12}$: 719.75

Sodium (2*S*,12*Z*,14*E*,16*S*,17*S*,18*R*,19*R*,20*R*,21*S*,22*S*,23*S*,24*E*)-21-(acetyloxy)-6,9,17,19-tetra-hydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-1,11-dioxo-1,2-dihydro-2,7-(epoxy-pentadeca[1,11,13]trienimino) naphtho[2,1-b]furan-5-olate [*14897-39-3*]

Rifamycin Sodium contains not less than 900 units (potency) per mg of rifamycin SV ($C_{37}H_{47}NO_{12}$: 697.77), calculated on the anhydrous basis.

Description Rifamycin Sodium appears as red, fine granular powder.
Rifamycin Sodium is freely soluble in methanol or in ethanol (95), soluble in water or in chloroform and practically insoluble in ether.

Identification (1) Dissolve 50 mg (potency) of Rifamycin Sodium in 50 mL of methanol. To 1 mL of this solution, add phosphate buffer (pH 7.0) to make 50 mL and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima at 314 nm and at 445 nm with the absorbance ratio being about 1.5.

(2) Dissolve 50 mg (potency) of Rifamycin Sodium in 10 mL of water. To 1 mL of this solution, add 1 mL of a 10 % solution of sodium persulfate in phosphate buffer (pH 7.0): a yellow precipitate is produced. Add 0.5 mL of 1 mol/L sodium carbonate solution to the precipitate after about 2 minutes: the precipitate dissolves completely and the solution shows a red-purple color.

(3) 1 % solution of Rifamycin Sodium responds to the Qualitative Tests (1) for sodium.

pH The pH of a solution obtained by dissolving 1 g (potency) of Rifamycin Sodium in 20 mL of water is between 6.5 and 7.5.

Absorbance $E_{1\,cm}^{1\%}$ (445 nm): 190 ~ 210 (anhydride). Weigh accurately about 20 mg (potency) of Rifamycin Sodium, dissolve in 5 mL of methanol and add a freshly prepared 0.1 % solution of *L*-ascorbic acid in phosphate buffer (pH 7.0) to make exactly 100 mL. Pipet 5 mL of this solution, add phosphate buffer (pH 7.0 to make exactly 50 mL and allow to stand for 30 minutes. Determine the absorbance at 445 nm of this solution as directed under Ultraviolet-visible Spectrophotometry.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Rifamycin Sodium according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Rifamycin B***—Weigh accurately about 1 g (potency) of Rifamycin Sodium, dissolve in acetone so that each mL contains 10 mg (potency) and use this solution as the test solution. Separately, weigh accurately about 20 mg of Rifamycin B RS, dissolve in acetone so that each mL contains 0.2 mg and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a solution prepared by dissolving 10 mg of *L*-ascorbic acid in a mixture of phosphate buffer (pH 7.0) and acetone (6:4) and air-dry the plate with protection from light. The yellow spot at the R_f value of about 0.5 obtained from the test solution is not larger or more intense than that from the standard solution. In the test solution, an orange spot appears at the R_f value of about 0.2 in addition to the R_f value of about 0.5.

Water 12.0 ~ 17.0 % (0.1 g, volumetric titration, direct titration).

Sterility Test It meets the requirement, when Rifamycin Sodium is used in a sterile preparation.

Bacterial Endotoxins Less than 0.50 EU/mg (potency) of rifamycin sodium, when used in a sterile preparation.

Abnormal Toxicity Dissolve 4 mg of Rifamycin Sodium in 0.5 mL of water for injection and inject intravenously for 15 to 30 seconds to each of 5 healthy mice weighing 17 g to 24 g. Use animals in which no abnormalities are observed for not less than 5 days

prior to the test. No animals die during the 24 hour post-dosage observation. If 1 animal dies, repeat the test with 5 animals: no animals die during the 24 hour observation.

Histamine It meets the requirement, when Rifamycin Sodium is used in a sterile preparation. Dissolve a suitable amount of Rifamycin Sodium in water so that each mL contains 30 mg (potency), use this solution as the test solution and use 0.1 mL of the solution for the test.

Assay ***The Cylinder-plate method*** (1) Agar media for seed and base layer- Use the culture medium in I 2 2) (2) ① under Microbial Assay for Antibiotics.

(2) Agar medium for transferring test organisms- Use the medium in I 2 2) (2) ① under Microbial Assay for Antibiotics.

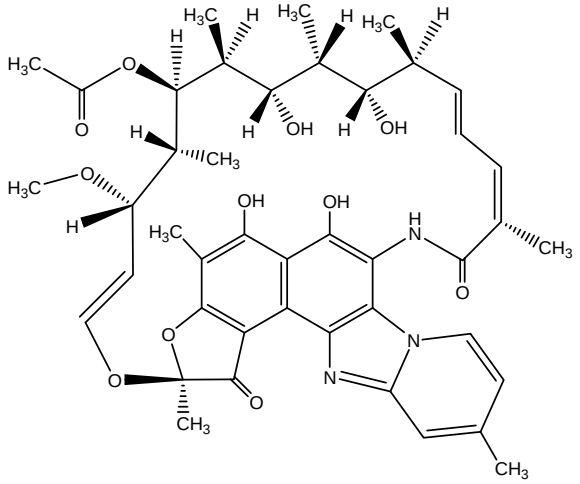
(3) Test organism and test organism suspension- Use *Micrococcus* NCTC 8340 as the test organism. Inoculate the test organism on the agar medium for transferring test organisms and incubate at 35 °C to 37 °C for 12 hours. Dilute the incubated organisms stepwise and perform the preliminary test to determine the quantity that displays the clearest inhibition zone. Add this amount to 100 mL of the agar medium for seed layer, previously melted and cooled to 48 °C, mix thoroughly and use this as the test organism suspension.

(3) Weigh accurately about 0.1 g (potency) of Rifamycin Sodium, dissolve in phosphate buffer (pH 7.0) to make exactly 100 mL and use this solution as the test stock solution. Pipet a suitable amount of the test stock solution, dilute with phosphate buffer (pH 7.0) so that each mL contains 1.0 units (potency) and 0.5 units (potency) and use these solutions as the high concentration test solution and the low concentration test solution, respectively. Separately, weigh accurately about 0.1 g (potency) of Rifamycin SV RS, dissolve in phosphate buffer (pH 7.0) to make exactly 100 mL and use this solution as the standard stock solution. Pipet a suitable amount of the standard stock solution, dilute with phosphate buffer (pH 7.0) so that each mL contains 1.0 units (potency) and 0.5 units (potency) and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively. Perform the test with these solutions as directed in I 8 under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and at a temperature between 2 °C to 8 °C.

Rifaximin



$C_{43}H_{51}N_3O_{11}$: 785.88

(2*S*,16*Z*,18*E*,20*S*,21*S*,22*R*,23*R*,24*R*,25*S*,26*R*,27*S*,28*E*)-5,6,21,23-Tetrahydroxy-27-methoxy-2,4,11,16,20,22,24,26-octamethyl-1,15-dioxo-1,2-dihydro-2,7-(Epoxypentadeca[1,11,13]trienimino) benzofuro[4,5-*e*]pyrido[1,2-*a*]benzimidazol-25-yl acetate [*80621-81-4*]

Rifaximin contains not less than 980 μg (potency) per mg of rifaximin ($C_{43}H_{51}N_3O_{11}$: 785.88), calculated on the anhydrous and solvent-free basis.

Description Rifaximin appears as orange powder. Rifaximin is soluble in methanol, in acetone, in chloroform or in ethyl acetate and practically insoluble in water.

Identification (1) Dissolve 15 mg (potency) of Rifaximin in methanol to make 10 mL. To 1 mL of this solution, add methanol to make 100 mL and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 453 nm and 457 nm, between 370 nm and 374 nm, between 291 nm and 295 nm and between 233 nm and 237 nm.

(2) Determine the infrared spectra of Rifaximin and Rifaximin RS as directed in the potassium bromide disk method under Infrared Spectrophotometery: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Dissolve about 50 mg (potency) each of Rifaximin and Rifaximin RS in 5 mL of methanol and use these solutions as the test solution and the standard solution, respectively. Perform the test with the test solution and the standard solution as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (95:5) and air-dry the plate.

Examine the plate under ultraviolet light (main wavelength: 254 nm): the spots obtained from the test solution and the standard solution show the same color and R_f value.

Crystalline Polymorphism Grind Rifaximin and Rifaximin RS with a glass rod until homogeneous. Pack into a X-ray powder diffractometer sample holder and perform the test as directed under X-ray Powder Diffraction Determination: the diffraction angle (2θ) characteristic of rifaximin α crystals is observed at <6.6°±0.2; 7.4°±0.2; 7.9°±0.2; 8.8°±0.2; 10.5°±0.2; 11.1°±0.2; 11.8°±0.2; 12.9°±0.2; 17.6°±0.2; 18.5°±0.2; 19.7°±0.2; 21.0°±0.2; 21.4°±0.2; 22.1°±0.2 >.

Operating conditions

X-ray tube: Copper anticathode

Wavelength: Kα1 (1.540562 Å) and Kα2 (1.544398 Å) (A nickel filter is used to suppress Kβ (1.392218 Å).

Detector: A sodium iodide scintillation counter

Tube current and voltage: 15 mA, 30 kV

Counting time: 1.3 seconds/0.02 °

Scan range of diffraction angle (2θ): 2.0 ~ 35.0 °

Sample holder: Amorphous glass (9200/2G of equal angles, 0.2 mm in depth)

Purity (1) ***Ethanol***—Weigh accurately about 1 g of Rifaximin, add 1 mL of the internal standard solution and *N,N*-dimethylformamide to make exactly 10 mL and use this solution as the test solution. Separately, weigh accurately 0.25 g of ethanol (95) and add *N,N*-dimethyl-formamide to make exactly 50 mL. Pipet 1 mL of this solution, add 1 mL of the internal standard solution and *N,N*-dimethylformamide to make exactly 10 mL and use this solution as the standard solution. Perform the test with 1 μL each of the test solution and the standard solution as directed under Gas Chromatography according to the following operating conditions and determine the ratios, Q_T and Q_S, of the peak area of ethanol to that of the internal standard (not more than 0.5 %).

$$\text{Content (\%) of ethanol} = \frac{Q_T}{Q_S} \times \frac{\text{Amount (mg) of ethanol taken}}{\text{Amount (mg) of Rifaximin taken}} \times \frac{100}{50}$$

Internal standard solution—To 1.5 g of 2-propanol, add *N,N*-dimethylformamide to make 100 mL.

Operating conditions

Detector: A hydrogen flame-ionization detector

Column: A tube about 0.53 mm in internal diameter and about 30 m in length, packed with diatomaceous earth for gas chromatography coated with dimethylpolysiloxane gum at the ratio of 100 %.

Column temperature: Keep at the initial temperature of 60 °C for 2 minutes, increase to 200 °C at the rate of 25 °C per minute and keep at 200 °C for 5 minutes.

Injection port and detector temperature: 220 °C

Carrier gas: Nitrogen

Flow rate: About 10 mL/minute

(2) ***Heavy metals***—Proceed with 1.0 g of Rifaximin according to Method 3 and peform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Weigh accurately about 0.1 g (potency) of Rifaximin, dissolve in 10 mL of acetonitrile, add water to make exactly 25 mL and use this solution as the test solution. Perform the test with 20 μL of the test solution as directed under Liquid Chromatography according to the following operating conditions. Determine each peak area by the automatic integration method and calculate the amount of each related substance by the area percentage method: the amount of rifaximin Y is not more than 0.5 %, the amount of each related substance is not more than 0.2 % and the total amount of related substances is not more than 2.0 %.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 276 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: 40 °C

Mobile phase: A mixture of mobile phase A and mobile phase B (63:37)

Mobile phase A: A mixture of methanol and acetonitrile (1:1)

Mobile phase B: 0.05 mol/L ammonium formate TS (adjust the pH to 7.2 with ammonia TS)

Flow rate: About 1.4 mL/minute

Relative retention time: Rifaximin Y 0.67

Time span of measurement: About 3 times as long as the retention time of rifaximin beginning after the solvent peak

Water Not more than 2.5 % (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately about 40 mg (potency) each of Rifaximin and Rifaximin RS and dissolve each in methanol to make exactly 100 mL. Pipet 10 mL each of these solutions, add 10 mL of the internal standard solution, add the mobile phase to make exactly 100 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 5 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the ratios, Q_T and Q_S, of the peak area of rifaximin to that of the internal standard in the test solution and the standard solution.

Amount [μg (potency)] of rifaximin ($C_{43}H_{51}N_3O_{11}$)

$$= \text{Amount [μg (potency)] of Rifaximin RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—Dissolve 45 mg of naproxen in methanol to make 100 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength 276 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octylsilyl silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of mobile phase A and mobile phase B (65:35)

Mobile phase A: A mixture of methanol and acetonitrile (60:40)

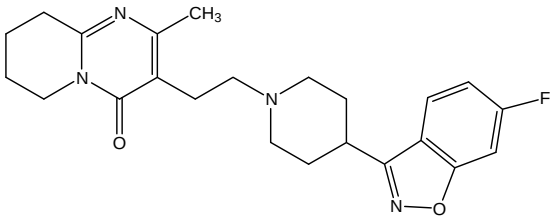
Mobile phase B: To 1000 mL of 0.01 mol/L ammonium dihydrogen phosphate, add 1 g of sodium heptanesulfonate and adjust the pH to 3.0 with acetic acid.

Flow rate: About 1.5 mL/minute

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Risperidone



$C_{23}H_{27}FN_4O_2$: 410.48

3-[2-[4-(6-Fluoro-1,2-benzoxazol-3-yl)piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-tetra-hydropyrido[1,2-a]pyrimidin-4-one [*106266-06-2*]

Risperidone contains not less than 99.0 % and not more than 101.0 % of risperidone ($C_{23}H_{27}FN_4O_2$), calculated on the dried basis.

Description Risperidone appears as white to pale yellow powder.

Risperidone is freely soluble in dichloromethane, sparingly soluble in alcohol, and practically insoluble in water.

Risperidone is soluble in dilute hydrochloric acid.

Risperidone shows crystalline polymorphism.

Identification (1) Determine the absorption spectra of the solution of Risperidone and Risperidone RS in 2-propanol (1 in 40000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Risperidone and Risperidone RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears between the spectra, dissolve each with the minimum possible volume of acetone, evaporate the solvent to dryness, and repeat the test with the residue.

Melting Point 169 ~ 173 °C.

Purity (1) ***Clarity and color of solution***—Dissolve 0.1 g of Risperidone in 0.75 w/v % of L-tartaric acid solution to make 100 mL: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 2.0 g of Risperidone according to Method 2 under Heavy Metals Limit Test and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Weigh accurately 0.10 g of Risperidone, add methanol to make exactly 100 mL and use this solution as the test solution. Pipet 1.0 mL of the test solution, and add methanol to make exactly 100 mL. Pipet 5.0 mL of the this solution, add methanol to make exactly 25 mL, and use this solution as the standard solution. Perform the test with 10 μL each of ethanol (blank), the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the area of the peaks from these solutions: the area of each peak other than the principal peak obtained from the test solution is not more than the area of the principal peak obtained from the standard solution and total area of the peaks other than the principal peak from the test solution is not more than 1.5 times the area of the principal peak from the standard solution. Disregard any peaks from the blank and any peaks having the area not more than 0.25 times the area of the principal peak from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 10 cm in length, packed with base-deactivated octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: 0.5 w/v % ammonium acetate solution.

Mobile phase B: Methanol

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-2	70	30

2-17	70→30	30→70
17-22	30	70

Flow rate: 1.5 mL/minute

System suitability

Test for required detectability: Pipet 2 mL of the standard solution and add methanol to make exactly 20 mL. Confirm that the peak area of risperidone obtained from 10 μL of this solution is equivalent to 7 % to 13 % of that from the standard solution

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of risperidone is not less than 1000 with the symmetry factor being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risperidone is not more than 2.0 %.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 4 hours).

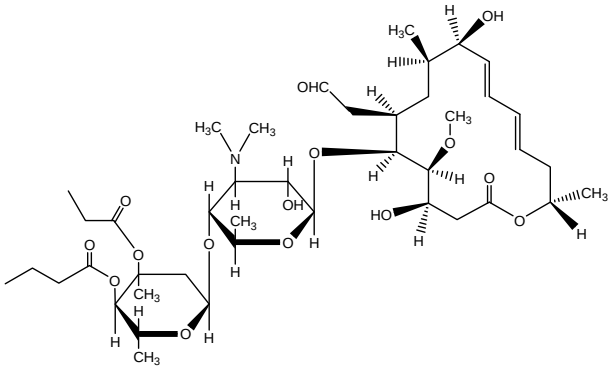
Residue on Ignition Not more than 0.1 % (1 g, platinum crucible).

Assay Weigh accurately about 0.16 g of Risperidone, add 70 mL of a mixture of 2-butanone and acetic acid (100) (7:1) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, endpoint detection method in titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 20.53 mg of $C_{23}H_{27}FN_4O_2$

Containers and Storage ***Containers***—Tight containers.

Rokitamycin



$C_{42}H_{89}NO_{15}$: 827.99

[(2*S*,3*S*,4*R*,6*S*)-6-[(2*R*,3*S*,4*R*,5*R*,6*S*)-6-[[(4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-4,10-Dihydroxy-5-methoxy-9,16-dimethyl-2-oxo-7-(2-oxo-ethyl)-1-oxacyclohexadeca-11,13-dien-6-yl]oxy]-4-(dimethylamino)-5-hydroxy-2-methyl-oxan-3-yl]oxy-2,4-dimethyl-4-propanoyloxy-oxan-3-yl] butanoate [*74014-51-0*]

Rokitamycin is a derivative of leucomycin A_5, which is a macrolide antibiotic produced by the growth of the mutants of *Streptomyces kitasatoensis*. Rokitamycin contains not less than 900 μg (potency) and not more than 1050 μg (potency) per mg of rokitamycin ($C_{42}H_{89}NO_{15}$: 827.99), calculated on the anhydrous basis.

Description Rokitamycin appears as white to yellow powder.

Rokitamycin is very soluble in methanol or in chloroform, freely soluble in ethanol (99.5) or in acetonitrile and praictically insoluble in water.

Identification (1) Determine the absorption spectra of the solutions of Rokitamycin and Rokitamycin RS in methanol (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Rokitamycin and Rokitamycin RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Determine the ^1H spectrum of a solution of Rokitamycin in deuterated chloroform for nuclear magnetic resonance spectroscopy (1 in 20), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy: it exhibits single signals A, B, C and D at around δ 1.4 ppm, at around δ 2.5 ppm, at around δ 3.5 ppm and at around δ 9.8 ppm, respectively. The ratio of integrated intensity of these signals, A:B:C:D, is about 3:6:3:1.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Rokitamycin according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Dissolve 50 mg of Rokitamycin in 50 mL of acetonitrile and use this solution as the test solution. Pipet 3 mL of the test solution, add acetonitrile to make exactly 100 mL and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine each peak area in each solution by the automatic integration method: the peak areas of 3”-*O*-propionylleucomycin A_7 having the relative retention time of about 0.72, 3”-*O*-propionyl-isoleucomycin A_5 having the relative retention time of about 0.86 and 3”-*O*-propionylleuco-

mycin A_1 having the relative retention time of about 1.36 with respect to rokitamycin from the test solution are not larger than the peak area of rokitamycin from the standard solution. Each peak area other than those of rokitamycin in the test solution, 3"-*O*-propionylleucomycin A_7, 3"-*O*-propionylisoleucomycin A_5 and 3"-*O*-propionylleucomycin A_1 is not larger than 23/100 times the peak area of rokitamycin in the standard solution, and the total area of peaks other than rokitamycin is not larger than 3 times the peak area of rokitamycin in the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 232 nm)

Column: A stainless steel column 4.0 mm in internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature ofa bout 55 °C

Mobile phase: A mixture of methanol, diluted 0.5 mol/L ammonium acetate TS (2 in 5) and acetonitrile (124:63:13)

Flow rate: Adjust the flow rate so that the retention time of rokitamycin is about 11 minutes.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution and add acetonitrile to make exactly 100 mL. Confirm that the peak area of rokitamycin obtained from 5 μL of this solution is equivalent to 7 % to 13 % of the peak area of rokitamycin from the standard solution.

System performance: When the procedure is run with 5 μL of the test solution under the above operating conditions, the number of theoretical plates of the peak of rokitamycin is not less than 3000 with the symmetry factor being not more than 1.5.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rokitamycin is not more than 2.0 %.

Time span of measurement: About 2.5 times as long as the retention time of rokitamycin, beginning after the solvent peak

Water Not more than 3.0 % (0.2, volumetric titration, direct titration).

Residue on Ignition Not more than 0.2 % (1 g).

Assay ***The Cylinder-plate method*** (1) Agar media for seed and base layer- Use the medium in I 2 1) (2) ② under Microbial Assay for Antibiotics. Adjust the pH of the medium so that it will be 7.8 to 8.0 after sterilization.

(2) Test organism- *Micrococcus luteus* ATCC 9341

(3) Weigh accurately an amount of Rokitamycin, equivalent to about 40 mg (potency), dissolve in 50 mL of methanol and add 0.1 mol/L phosphate buffer (pH 4.5) to make exactly 100 mL. Pipet a suitable amount of this solution, dilute with 0.1 mol/L phosphate buffer (pH 8.0) containing 0.01 % of polysorbate 80 to make solutions containing 2 μg (potency) and 0.5 μg (potency) per mL and use these solutions as the high concentration test solution and the low concentration test solution, respectively. Separately, weigh accurately an amount of Rokitamycin RS, equivalent to about 40 mg (potency), dissolve in 50 mL of methanol, add 0.1 mol/L phosphate buffer (pH 4.5) to make exactly 100 mL and use this solution as the standard stock solution. Keep the standard stock solution at 5 °C or below and use within 10 days. Pipet a suitable amount of the standard stock solution, dilute with 0.01 mol/L phosphate buffer (pH 8.0) containing 0.01 % of polysorbate 80 to make solutions containing 2 μg (potency) and 0.5 μg (potency) per mL and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively. Perform the test with these solutions as directed in I 8 under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Rokitamycin Tablets

Rokitamycin Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of rokitamycin ($C_{42}H_{89}NO_{15}$: 827.99).

Method of Preparation Prepare as directed under Tablets, with Rokitamycin.

Identification Weigh an amount of powdered Rokitamycin Tablets, equivalent to 10 mg (potency) of rokitamycin according to the labeled amount, add 20 mL of methanol and centrifuge, if necessary. To 1 mL of this solution, add methanol to make 25 mL and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 230 nm and 233 nm.

Dissolution Test Perform the test with 1 tablet of Rokitamycin Tablets at 50 revolutions per minute according to Method 2 under Dissolution Test, using 900 mL of water as the dissolution solution. Take not less than 20 mL of the dissolved solution after 30 minutes from the start of the test and filter through a membrane filter with a pore size of not more than 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 22 μg (potency) of rokitamycin per mL according to the labeled amount and use this solution as the test solution. Separately, weigh accurately an amount of Rokitamycin RS,

equivalent to about 22 mg (potency), dissolve in 10 mL of methanol and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 232 nm of the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry, using water as the blank. The dissolution rate of Rokitamycin Tablets in 30 minutes is not less than 80 %.

Dissolution rate (%) with respect to the labeled amount of rokitamycin ($C_{42}H_{69}NO_{15}$) = Amount [mg (potency)] of Rokitamycin RS $\times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 90$

C: Labeled amount [mg (potency)] of rokitamycin ($C_{42}H_{69}NO_{15}$) in 1 tablet

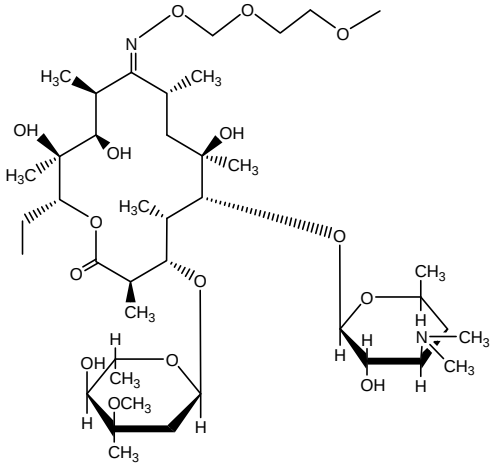
Uniformity of Dosage Units It meets the requirement when the content uniformity test is performed according to the following method.
Add 50 mL of water to 1 tablet of Rokitamycin Tablets and disintegrate. Add 10 mL of methanol, shake well and add water to make exactly 100 mL. Centrifuge this solution if necessary and filter through a membrane filter with a pore size of not more than 0.5 μm. Discard the first 5 mL of the filtrate, pipet *V* mL of the subsequent filtrate, add water to make exactly *V'* mL so that each mL contains about 20 μg (potency) of rokitamycin and use this solution as the test solution. Separately, weigh accurately about 20 mg (potency) of Rokitamycin RS, dissolve in 10 mL of methanol and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 232 nm of the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry, using water as the blank.

Amount [mg (potency)] of rokitamycin ($C_{42}H_{69}NO_{15}$) = Amount [mg (potency)] of Rokitamycin RS $\times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{10}$

Assay Proceed as directed in the Assay under Rokitamycin. Weigh accurately and powder not less than 20 Rokitamycin Tablets. Weigh accurately a portion of the powder, equivalent to about 40 mg (potency) of rokitamycin, add 50 mL of methanol, shake vigorously, add 0.01 mol/L phosphate buffer (pH 4.5) to make exactly 100 mL and centrifuge, if necessary. Pipet a suitable amount of this solution, dilute with a solution prepared by adding 0.1 mol/L phosphate buffer (pH 8.0) to 0.1 g of polysorbate 80 to make 1000 mL, so that each mL contains 2 μg (potency) and 0.5 μg (potency) and use these solutions as the high concentration test solution and the low concentration test solution, respectively.

Containers and Storage ***Containers***—Tight containers.

Roxithromycin



$C_{41}H_{76}N_2O_{15}$: 837.05

(3*R*,4*S*,5S,6*R*,7R,9*R*,10*Z*,11*S*,12*R*,13*S*,14*R*)-6-[(2*S*,3*R*,4S,6*R*)-4-(Dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-14-ethyl-7,12,13-trihydroxy-4-[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-10-(2-methoxyethoxymethoxyimino)-3,5,7,9,11,13-hexamethyl-oxacyclotetradecan-2-one [*80214-83-1*]

Roxithromycin is a derivative of erythromycin.
Roxithromycin contains not less than 970 μg (potency) and not more than 1020 μg (potency) per mg of roxithromycin ($C_{41}H_{76}N_2O_{15}$), calculated on the anhydrous basis.

Description Roxithromycin is white crystalline powder.
Roxithromycin is freely soluble in ethanol (95) or in acetone, soluble in methanol, sparingly soluble in acetonitrile and practically insoluble in water.

Identification Determine the infrared spectra of Roxithromycin and Roxithromycin RS, as directed in the potassium bromide disk method under Infrared spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: -93 ~ -96° (0.5 g calculated on the anhydrous basis, acetone, 50 mL, 100 mm).

Purity (1) ***Heavy metals***—Proceed with 2 g of Roxithromycin according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Erythromycin and erythromycin oxime***— Weigh accurately 0.2 g (potency) of Roxithromycin, dissolve in methanol, make to exactly 10 mL and use this solution as the test solution. Separately, weigh accurately 40 mg (potency) of Erythromycin RS, dissolve in methanol, make to exactly 100 mL and use this solution as the erythromycin standard solution. Weigh accurately 40 mg of Erythromycin oxime RS, dissolve in methanol, make to exactly 100 mL and use this solution as the erythromycin oxime standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL of each solution on a plate of silica gel for thin-layer chromatography, develop the plate with the mixture of toluene, chloroform and diethylamine (50:40:7) as the developing solvent mixture, and dry the plate at 100 ~ 105 °C for 5 minutes. Spray evenly the color developing solution on the plate and heat the plate at 100 to 105 °C. The color developing solution mixture contains 2.5 g of phosphomolybdic acid *n*-hydrate, 2.5 mL of sulfuric acid and 50mL of acetic acid (100). The spots obtained from the test solution are not larger or more intense than the spot from the erythromycin standard solution (R_f value: about 0.35) and the spot from the erythromycin oxime standard solution (R_f value: about 0.28) (Each is not more than 2.0 %).

(3) ***Related substances***—Weigh accurately 40 mg of Roxithromycin, dissolve in mobile phase A to make exactly 10 mL and use this solution as the test solution. Separately, weigh accurately about 20 mg of Roxithromycin RS and dissolve in mobile phase A to make exactly 10 mL. Pipet 1 mL of this solution, add mobile phase A to make exactly 100 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine each peak area in each solution by the automatic integration method: the area of a peak having the relative retention time of about 1.05 with respect to roxithromycin from the test solution is no larger than 2 times the peak area of roxithromycin from the standard solution. The area of each peak other than the peak of roxithromycin and the peak having the relative retention time of about 1.05 is not larger than the peak area of roxithromycin from the standard solution, and the total peak areas other than roxithromycin from the test solution is not larger than 6 times the peak area of roxithromycin from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 205 nm)

Column: A stainless steel column 4.6 mm in internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Control the concentration gradient by mixing mobile phases A and B as directed in the following table.

Mobile phase A: To 200 mL of ammonium dihydrogen phosphate solution (17 in 100), add 510 mL of water and adjust the pH to 5.3 with 2 mol/L sodium hydroxide TS. To this solution, add 315 mL of acetonitrile.

Mobile phase B: A mixture of acetonitrile and water (7:3)

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-38	100	0
38-39	100→90	0→10
39-80	90	10

Flow rate: Adjust the flow rate so that the retention time of roxithromycin is about 21 minutes.

System suitability

Test for required detectability: Pipet 2 mL of the standard solution and add mobile phase A to make exactly 10 mL. Confirm that the peak area of roxithromycin obtained from 20 μL of this solution is equivalent to 15 % to 25 % of that from 20 μL of the standard solution.

System performance: Dissolve 5 mg each of Roxithromycin RS and *N*-demethylroxithromycin in mobile phase A to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, *N*-demethylroxithromycin and roxithromycin are eluted in this order with the resolution between their peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of roxithromycin is not more than 2.0 %.

Water Not more than 3.0 % (0.3 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately an amount of Roxithromycin and Roxithromycin RS, equivalent to about 20 mg (potency), dissolve each in the mobile phase and make exactly 10 mL, and use these solutions as the test solution and the standard solution. Perform the test with exactly 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of Roxithromycin.

$$\text{Amount [μg (potency)] of roxithromycin } (C_{41}H_{76}N_2O_{15}) = \text{Amount [μg (potency)] of Roxithromycin RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 205 nm)

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: To 200 mL of ammonium dihydrogen phosphate solution (17 in 100), add 510 mL of water and adjust the pH to 5.3 with 2 mol/L sodium hydroxide TS. To this solution, add 315 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of roxithromycin is about 11 minutes.

System suitability

System performance: Dissolve 5 mg each of Roxithromycin RS and *N*-demethylroxithromycin in the mobile phase to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, *N*-demethylroxithromycin and roxithromycin are eluted in this order with the resolution between their peaks being not less than 6 and the symmetry factor of the peak of roxithronycin being not more than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of roxithromycin is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Saccharated Pepsin

Saccharated Pepsin is an enzyme with protein digestibility and is prepared by mixing lactose hydrate into pepsin obtained from stomach mucosa of swine or bovis.

1 g of Saccharated Pepsin contains not less than 3800 units and not more than 6000 units.

Description Saccharated Pepsin appears as white powder, and has a characteristic odor and a slightly sweet taste.

Saccharated Pepsin is soluble in water with slight turbidity and insoluble in ethanol (95) or in ether.

Saccharated Pepsin is slightly hygroscopic.

Purity (1) ***Rancidity***—Saccharated Pepsin doses not have a unpleasant or deteriorated odor.

(2) ***Acid***—Dissolve 0.5 g of Saccharated Pepsin in 50 mL of water and add 0.50 mL of 0.1 mol/L sodium hydroxide VS and 2 drops of phenolphthalein TS: a red color develops.

Loss of Drying Not more than 1.0 % (1 g, 80 °C, 4 hours).

Residue on Ignition Not more than 0.5 % (1 g).

Assay (1) Test solution Weigh accurately an amount of Saccharated Pepsin equivalent to about 1250 units, dissolve in ice-cold 0.01 mol/L hydrochloric acid TS to make exactly 50 mL.

(2) Standard solution Weigh accurately a suitable amount of Saccharated Pepsin RS and dissolve in 0.01 mol/L hydrochloric acid TS to make a solution containing about 25 units per mL

(3) Substrate solution Use the substrate solution 1 described in (2) Assay for protein digestive activity under the Digestion Test. After adjusting pH to 2.0.

(4) Procedure Proceed with the test solution as directed in (2) Assay for protein digestive activity under the Digestion Test and determine the absorbances, A_T and A_{TB}, using trichloroacetic acid TS A as the precipitation reagent. Separately, determine the absorbances, A_S and A_{SB}, of the standard solution in the same manner as the test solution. Calculate the unit in 1 g of Saccharated Pepsin as the following equation.

$$\text{Units in 1 g of Saccharated Pepsin} = U_S \times \frac{(A_T - A_{TB})}{(A_S - A_{SB})} \times \frac{1}{W}$$

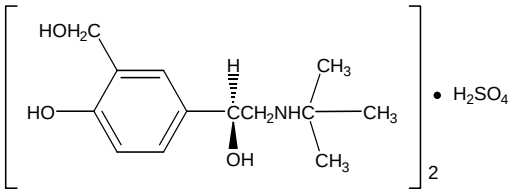
U_S: Units per 1 mL of the standard solution,

W: Amount (g) of Saccharated Pepsin per 1 mL of the test solution.

Containers and Storage ***Containers***—Tight containers.

Storage—Not exceeding 30 °C.

Salbutamol Sulfate



and enantiomer

$(C_{13}H_{21}NO_3)_2 \cdot H_2SO_4$: 576.70

Bis{*N*-*t*-butyl-2-hyroxy-2-[4-hydroxy-(3-hydroxymethyl)phenyl]ethanamine} sulfate [*51022-70-9*]

Salbutamol Sulfate, when dried, contains not less than 98.0 % and not more than 101.0 % of salbutamol sulfate $[(C_{13}H_{21}NO_3)_2 \cdot H_2SO_4]$.

Description Salbutamol Sulfate appears as white powder.

Salbutamol Sulfate is freely soluble in water, slightly

soluble in ethanol (95) or in acetic acid (100) and practically insoluble in ether.
A solution of Salbutamol Sulfate (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectra of the solutions of Salbutamol Sulfate and Salbutamol Sulfate RS in 0.1 mol/L hydrochloric acid TS (1 in 12500) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Salbutamol Sulfate and Salbutamol Sulfate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Salbutamol Sulfate (1 in 20) responds to the Qualitative Tests for sulfate.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Salbutamol Sulfate in 20 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Salbutamol Sulfate according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of the standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Dissolve 20 mg of Salbutamol Sulfate in 10 mL of water and use this solution as the test solution. Pipet 1 mL of the test solution, add water to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 2-propanol, water and ammonia solution (28) (25: 15 : 8 : 2) to a distance of about 15 cm and air-dry the plate. Leave the plate in a well-closed vessel saturated with diethylamine vapor for 5 minutes and spray evenly 4-nitrobenzenediazonium hydrochloride TS for spraying: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution in color.

(4) ***Boron***—Take 50 mg of Salbutamol Sulfate and 5.0 mL of the boron standard solution and transfer to a platinum crucible, respectively. Add 5 mL of potassium carbonate-sodium carbonate TS, evaporate on a water-bath to dryness, dry at 120 °C for 1 hour and ignite the residue immediately. After cooling, add 0.5 mL of water and 3 mL of curcumin TS to the residue and warm gently in a water-bath for 5 minutes. After cooling, add 3 mL of acetic acid (100)-sulfuric acid TS, mix and allow to stand for 30 minutes. Add ethanol (95) to make exactly 100 mL and filter. Discard the first 10 mL of the filtrate and use the subsequent filtrate as the test solution and the standard solution, respectively. Perform the test with the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry, using ethanol (95) as the blank: the absorbance of the test solution at 555 nm is not larger than that of the standard solution.

Loss on Drying Not more than 0.5 % (1 g, not more than 0.67 kPa, 100 °C, 3 hours).

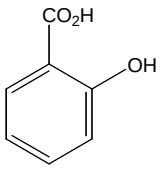
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.9 g of Salbutamol Sulfate, previously dried and dissolve in 50 mL of acetic acid (100) by warming. After cooling, titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 3 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 57.67 mg of $(C_{13}H_{21}NO_3)_2 \cdot H_2SO_4$

Containers and Storage ***Containers***—Tight containers.

Salicylic Acid



$C_7H_6O_3$: 138.12

2-Hydroxybenzoic acid [*69-72-7*]

Salicylic Acid, when dried, contains not less than 99.5 % and not more than 101.0 % of salicylic acid ($C_7H_6O_3$).

Description Salicylic Acid appears as white crystals or crystalline powder, is odorless and has a slightly acidic, followed by an acrid taste.
Salicylic Acid is freely soluble in ethanol (95), in acetone or in ether, soluble in hot water and slightly soluble in water.

Identification (1) A solution of Salicylic Acid (1 in 500) responds to the Qualitative Tests (1) and (3) for salicylate.

(2) Determine the absorption spectra of solutions of Salicylic Acid and Salicylic Acid RS in ethanol (95) (3 in 200000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Salicylic Acid and Salicylic Acid RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting Point 158 ~ 161 °C.

Purity (1) ***Chloride***—Dissolve 5.0 g of Salicylic Acid in 90 mL of water by heating, cool, dilute with water to make 100 mL and filter. Discard the first 20 mL of the filtrate, take subsequent 30 mL of the filtrate, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test. Prepare the control solution with 0.35 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.008 %).

(2) ***Sulfate***—To 30 mL of the filtrate obtained in (1), add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.011 %).

(3) ***Heavy metals***—Dissolve 2.0 g of Salicylic Acid in 25 mL of acetone, add 4 mL of sodium hydroxide, 2 mL of dilute acetic acid and water to make 50 mL and Perform the test. Prepare the control solution as follows: to 2.0 mL of standard lead solution, add 25 mL of acetone, 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(4) ***Related substances***—Dissolve 0.50 g of Salicylic Acid in the mobile phase to make exactly 100 mL, and use this solution as the test solution. Separately, weigh accurately 10 mg of phenol, 25 mg of 4-hydroxy-isophthalic acid, and 50 mg of parahydroxybenzoic acid, and dissolve in the mobile phase to make exactly 100 mL. To 1 mL of this solution add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of parahydroxybenzoic acid, 4-hydroxyisophthalic acid, and phenol from the test solution are not larger than the area of each respective peak from the standard solution, the area of the peak other than salicylic acid from the test solution is not larger than the peak area of 4-hydroxyisophthalic acid from the standard solution, and the total area of the peaks other than salicylic acid is not larger than 2 times the peak area of parahydroxybenzoic acid from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 270 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35 °C

Mobile phase: A mixture of water, methanol, and acetic acid (100) (60 : 40 : 1)

Flow rate: Adjust the flow rate so that the retention time of salicylic acid is about 17 minutes.

System suitability

Test for required detectability: Pipet 2 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak areas of parahydroxybenzoic acid, 4-hydroxyisophthalic acid, and phenol obtained from 10 μL of this solution is equivalent to 14 to 26 % of the area of each respective peak from 10 μL of the standard solution.

System performance: Dissolve 10 mg of phenol, 25 mg of 4-hydroxyisophthalic acid, and 50 mg of parahydroxybenzoic acid in 100 mL of the mobile phase. To 1 mL of this solution add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. When the procedure is run under the above operating conditions, parahydroxybenzoic acid, 4-hydroxy-isophthalic acid, and phenol are eluted in this order with the resolution between the peaks of 4-hydroxyisophthalic acid and phenol being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of parahydroxybenzoic acid, 4-hydroxyisophthalic acid, and phenol is not more than 2.0 %, respectively.

Time span of measurement: About 2 times as long as the retention time of the salicylic acid beginning after the solvent peak.

(5) ***Readily carbonizable substances***—Perform the test with 0.5 g of Salicylic Acid: the solution has no more color than Color Matching Fluid C.

Loss on Drying Not more than 0.5 % (2 g, silica gel, 3 hours).

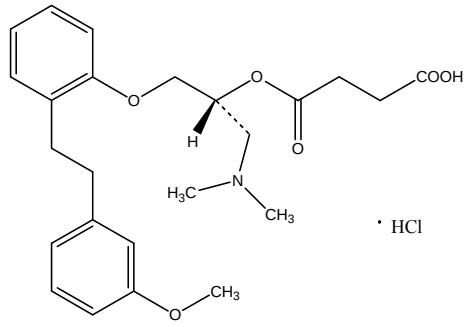
Residue on Ignition Not more than 0.05 % (1 g).

Assay Weigh accurately about 0.5 g of Salicylic Acid, previously dried, dissolve in 25 mL of neutralized ethanol and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 13.812 mg of $C_7H_6O_3$

Containers and Storage ***Containers***—Well-closed containers.

Sarpogrelate Hydrochloride



and enantiomer

$C_{24}H_{31}NO_6 \cdot HCl$: 465.97

1-[2-(Dimethylamino)-1-[[2-[2-(3-methoxyphenyl)ethyl]phenoxy]methyl]ethyl hydrogen butanedioate hydrochloride [*135159-51-2*]

Sarpogrelate Hydrochloride contains not less than 98.5 % and not more than 101.0 % of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6 \cdot HCl$: 465.97), calculated on the anhydrous basis.

Description Sarpogrelate Hydrochloride appears as white crystalline powder.
Sarpogrelate Hydrochloride is slightly soluble in water and in ethanol (95).
Sarpogrelate Hydrochloride dissolves in 0.01 mol/L hydrochloric acid TS.
A solution of Sarpogrelate Hydrochloride (1 in 100) shows no optical rotation.
Sarpogrelate Hydrochloride exhibits crystalline polymorphism.

Identification (1) Determine the absorption spectra of solutions of Sarpogrelate Hydrochloride and Sarpogrelate Hydrochloride RS in 0.01 mol/L hydrochloric acid TS (1 in 20000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Sarpogrelate Hydrochloride and Sarpogrelate Hydrochloride RS as directed in the potassium chloride disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Dissolve 0.3 g of Sarpogrelate Hydrochloride in 6 mL of sodium hydroxide TS, shake well, allow to stand for 10 minutes, and filter. To 1 mL of the filtrate, add 1 mL of dilute nitric acid: the solution responds to the Qualitative Tests for chloride.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Sarpogrelate Hydrochloride according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Arsenic***— Prepare the test solution with 2.0 g of Sarpogrelate Hydrochloride according to Method 4 and perform the test. (not more than 1 ppm).

(3) ***Related substances***—Carry out this procedure within 3 hours after preparing the test solution. Dissolve 20 mg of Sarpogrelate Hydrochloride in 10 mL of the mobile phase, use this solution as the test solution. Pipet 2 mL of this solution, add the mobile phase to make exactly 200 mL, use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the peak area of decomposed substance A, having the relative retention time about 0.82 to sarpogrelate, obtained from the test solution is not larger than 1/5 times that of sarpogrelate from the standard solution; the area of the peak other than sarpogrelate and the peak mentioned above from the test solution is not larger than 1/10 times the peak area of sarpogrelate from the standard solution; the total area of the peaks other than sarpogrelate from the test solution is not larger than 1/2 times the peak area of sarpogrelate from the standard solution. For this calculation, use the peak area of the decomposed substance A, having the relative retention time about 0.82 to sarpogrelate, after multiplying by the relative response factor, 0.78.

Operating conditions

Detector, column, column temperature, mobile phase, flow rate: Proceed as directed in the operating conditions in the Assay

System suitability

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of sarpogrelate with 10 μL of this solution is equivalent to 7 to 13 % of the peak area of sarpogrelate with the standard solution.

System performance: Dissolve 50 mg of Sarpogrelate Hydrochloride in 20 mL of water, use this solution as the sarpogrelate hydrochloride stock solution. To 1 mL of this solution add 2 mL of sodium hydroxide TS, shake thoroughly, allow to stand for 10 minutes, and add 3 mL of 1 mol/L hydrochloric acid TS. To this solution add 1 mL of the sarpogrelate hydrochloride stock solution, and add the mobile phase to make 50 mL. When the procedure is run with 10 mL of this solution under the above operating conditions, the decomposed substance A and sarpogrelate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sarpogrelate is not more than 2.0 %.

Time span of measurement: About 2.5 times as long as the retention time of sarpogrelate beginning after the solvent peak.

Water Not more than 0.5 % (0.1 g, coulometric titration).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 50 mg each of Sarpogrelate Hydrochloride and Sarpogrelate Hydrochloride RS (separately determine the water in the same manner as Sarpogrelate Hydrochloride) add to them exactly 2.5 mL each of the internal standard solution, and dissolve in the mobile phase to make 50 mL. Pipet 5 mL each of these solutions, add the mobile phase to make 50 mL, use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of sarpogrelate to that of the internal standard.

$$\text{Amount (mg) of sarpogrelate hydrochloride } (C_{24}H_{31}NO_6 \cdot HCl) = W_S \times \frac{Q_T}{Q_S}$$

W_S: Amount (mg) of Sarpogrelate Hydrochloride RS taken, calculated on the dried basis.

Internal standard solution—A solution of isopropyl paraoxybenzoate in the mobile phase (3 in 1000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 272 nm).

Column: A stainless steel column about 4.6 nm in internal diameter and about 15 cm in length, packed with octadecylsilanized silicagel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: A mixture of water, acetonitrile and trifluoroacetic acid (1300 : 700 : 1).

Flow rate: Adjust the flow rate so that the retention time of sarpogrelate is about 8 minutes.

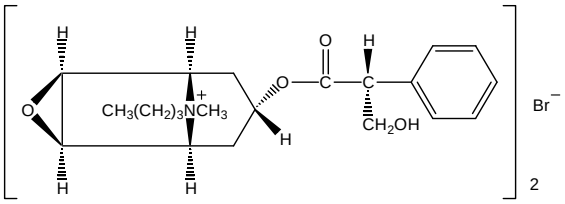
System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, sarpogrelate and the internal standard substance are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sarpogrelate to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Scopolamine Butylbromide



Hyoscine Butylbromide $C_{21}H_{30}BrNO_4$: 440.37

(1*S*,2*S*,4*R*,5*R*,7*S*)-9-Butyl-7-[(2*S*)-3-hydroxy-2-phenylpropanoyloxy]-9-methyl-3-oxa-9-azoniatricyclo[3.3.1.$0^{2,4}$]nonane bromide [*149-64-4*]

Scopolamine Butylbromide, when dried, contains not less than 98.5 % and not more than 101.0 % of scopolamine butylbromide ($C_{21}H_{30}BrNO_4$).

Description Scopolamine Butylbromide appears as white crystals or crystalline powder.

Scopolamine Butylbromide is very soluble in water, freely soluble in acetic acid (100), soluble in ethanol (95), sparingly soluble in methanol, slightly soluble in acetic anhydride and practically insoluble in ether.

Melting point—About 140 °C (with decomposition).

Identification (1) To 1 mg of Scopolamine Butylbromide, add 3 to 4 drops of fuming nitric acid and evaporate on a water-bath to dryness. After cooling, dissolve the residue in 1 mL of *N,N*-dimethylformamide and add 6 drops of tetraethylammonium hydroxide TS: a red-purple color develops.

(2) Determine the absorption spectra of the solutions of Scopolamine Butylbromide and Scopolamine Butylbromide RS (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Scopolamine Butylbromide and Scopolamine Butylbromide RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(4) A solution of Scopolamine Butylbromide (1 in 20) responds to the Qualitative Tests for bromide.

pH Dissolve 1.0 g of Scopolamine Butylbromide in 10 mL of water: the pH of this solution is between 5.5 and 6.5.

Specific Optical Rotation $[\alpha]_D^{20}$: -18.0 ~ -20.0° (after drying, 1 g, water, 10 mL, 100 mm).

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Scopolamine Butylbromide in 10 mL of water: the solution is clear and has no more color than the following control solution.

Control solution—To 0.5 mL of Color Matching Fluid F, add diluted hydrochloric acid (1 in 40) to make 20 mL.

(2) ***Heavy metals***—Proceed with 2.0 g of Scopolamine Butylbromide according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Dissolve 0.10 g of Scopolamine Butylbromide in the mobile phase to make exactly 10 mL and use this solution as the test solution. Separately, dissolve 10 mg of Scopolamine Hydrobromide RS in the mobile phase to make exactly 100 mL. Pipet 10.0 mL of this solution. add the mobile phase to make exactly 50 mL and use this solution as the standard solution (1). Pipet 5.0 mL of the standard solution (1), add the mobile phase to make exactly 10 mL and use this solution as the standard solution (2). Perform the test with 20 μL each of the test solution and the standard solutions (1) and (2) as directed under Liquid Chromatography according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the peak area of scopolamine from the test solution is not larger than that from the standard solution (2) and each area of the peaks other than the peak appearing in the first and the peak of scopolamine and butylscopolamine from the test solution are not larger than the peak area from the standard solution (1).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column, about 4 mm in internal diameter and 15 cm to 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 30 °C.

Mobile phase: Dissolve 2 g of sodium lauryl sulfate in 370 mL of water and 680 mL of methanol and adjust with diluted phosphoric acid (1 in 10) to a pH of 3.6.

Flow rate: Adjust the flow rate so that the retention time of butylscopolamine is about 7 minutes.

System suitability

Detection sensitivity: Adjust the detection sensitivity so that the peak height of scopolamine from 20 μL of the standard solution (2) is between 5 mm and 10 mm.

System performance: Dissolve 5 mg each of Scopolamine Butylbromide and Scopolamine Hydrobromide in 50 mL of mobile phase. When the procedure is run with with 20 μL of this solution under the above operating conditions, scopolamine and scopolamine butylbromide are eluted in this order with a resolution between their peaks being not less than 5.0.

Time span of measurement: About twice as long as the retention time of butylscopolamine.

Loss on Drying Not more than 1.0 % (1 g, 105 °C, 4 hours).

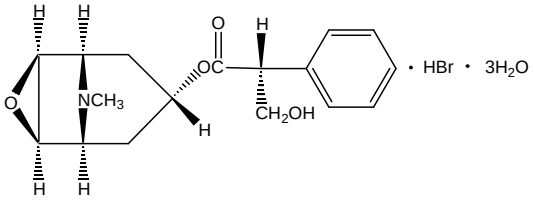
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately 0.8 g of Scopolamine Butylbromide, previously dried, dissolve in 40 mL of acetic acid (100) and 30 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 44.04 mg of $C_{21}H_{30}BrNO_4$

Containers and Storage ***Containers***—Tight containers.

Scopolamine Hydrobromide Hydrate



$C_{17}H_{21}NO_4 \cdot HBr \cdot 3H_2O$: 438.31

(1*S*,2*S*,4*R*,5*R*,7*S*)-9-Methyl-3-oxa-9-azatricyclo[3.3.1.02,4]non-7-yl (2*S*)-3-hydroxy-2-phenylpropanoate hydrobromide trihydrate [*6533-68-2*]

Scopolamine Hydrobromide Hydrate, when dried, contains not less than 98.5 % of scopolamine hydrobromide ($C_{17}H_{21}NO_4 \cdot HBr$: 384.27).

Description Scopolamine Hydrobromide Hydrate appears as colorless or white crystals, or white granules or powder and is odorless.
Scopolamine Hydrobromide Hydrate is freely soluble in water, sparingly soluble in acetic acid (100) or in ethanol (95) and practically insoluble in ether.

Identification (1) To 1 mg of Scopolamine Hydrobromide Hydrate, add 3 to 4 drops of fuming nitric acid, evaporate on a water-bath to dryness and cool. Dissolve the residue in 1 mL of *N,N*-dimethylformamide and add 6 drops of

tetraethylammonium hydroxide TS: a red-purple color is produced.

(2) A solution of Scopolamine Hydrobromide Hydrate (1 in 20) responds to the Qualitative Tests for bromide.

Specific Optical Rotation $[\alpha]_D^{20}$: -24.0 ~ -26.0° (after drying, 0.5 g, water, 10 mL, 100 mm).

Melting Point 195 ~ 199 °C (after drying, previously heat the bath to 180 °C).

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Scopolamine Hydrobromide Hydrate in 10 mL of water: the solution is clear and colorless.

(2) ***Acid***—Dissolve 0.50 g of Scopolamine Hydrobromide Hydrate in 15 mL of water and add 0.50 mL of 0.02 mol/L sodium hydroxide VS and 1 drop of methyl red-methylene blue TS: a green color develops.

(3) ***Apoatropine***—Dissolve 0.20 g of Scopolamine Hydrobromide Hydrate in 20 mL of water, add 0.60 mL of 0.002 mol/L potassium permanganate VS and allow to stand for 5 minutes: the red color in the solution does not disappear.

(4) ***Related substances***—Dissolve 0.15 g of Scopolamine Hydrobromide Hydrate in 3 mL of water and use this solution as the test solution.

(i) To 1 mL of the test solution, add 2 to 3 drops of ammonia TS: no turbidity is produced.

(ii) To 1 mL of the test solution, add 2 to 3 drops of potassium hydroxide TS: a transient white turbidity might be produced and disappears clearly in a little while.

Loss on Drying Not more than 13.0 % [1.5 g: first dry in a desiccator (silica gel) for 24 hours, then dry at 105 °C for 3 hours].

Residue on Ignition Not more than 0.1 % (1 g).

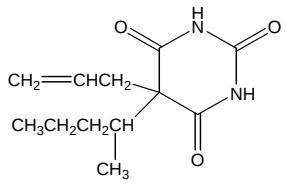
Assay Weigh accurately about 0.5 g of Scopolamine Hydrobromide Hydrate, previously dried and dissolve in 10 mL of acetic acid (100) by warming. After cooling, add 40 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 38.426 mg of $C_{17}H_{21}NO_4 \cdot HBr$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Secobarbital



$C_{12}H_{18}N_2O_3$: 238.28

5-(Pentan-2-yl)-5-(prop-2-en-1-yl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione [*76-73-3*]

Secobarbital contains not less than 97.5 % and not more than 100.5 % of scobarbital ($C_{12}H_{18}N_2O_3$), calculated on the dried basis.

Description Secobarbital is a white amorphous or crystalline powder, is odorless and has a slightly bitter taste.

Secobarbital is very slightly soluble in water, freely soluble in ethanol (95) or in ether and soluble in chloroform.

Secobarbital dissolves in sodium hydroxide TS or sodium carbonate TS.

pH—The pH of the saturated solution of Secobarbital is about 5.6.

Identification Determine the infrared spectra of Secobarbital and Secobarbital RS, as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity ***Isomer content***— Weigh accurately about 0.3 g of Secobarbital, dissolve in 5 mL sodium hydroxide solution (1 in 100), add 300 ± 5 mg of a solution of 4-nitrobromobenzyl in 10 mL of ethanol (95), reflux for 30 minutes, cool, collect the precipitation on a small filter, wash with water and recrystallize the precipitate from 25 mL of ethanol (95) and dried at 105 °C for 30 minutes; the precipitate melts between 156 °C and 161 °C.

Loss on Drying Not more than 1.0 % (1 g, silica gel, 18 hours)

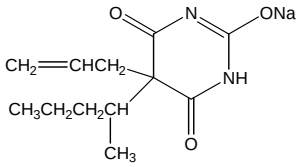
Residue on Ignition Not more than 0.1 % (1 g)

Assay Weigh accurately about 0.45 g of Secobarbital and dissolve in 60 mL of dimethylformamide. Titrate with 0.1 mol/L sodium methoxide VS, taking precautions against the absorption of atmospheric carbon dioxide (Indicator: 4 drops of thymol blue TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium methoxide VS
= 23.828 mg of $C_{12}H_{18}N_2O_3$

Containers and Storage ***Containers***—Tight containers.

Secobarbital Sodium



$C_{12}H_{17}N_2NaO_3$: 260.27

4,6-Dioxo-5-(pentan-2-yl)-5-(prop-2-en-1-yl)-1,4,5,6-tetrahydropyrimidin-2-olate [*309-43-3*]

Secobarbital Sodium contains not less than 98.5 % and not more than 100.5 % of secobarbital sodium ($C_{12}H_{17}N_2NaO_3$), calculated on the dried basis.

Description Secobarbital Sodium appears as white powder, is ordorless and has a bitter taste.
Secobarbital Sodium is very soluble in water, soluble in ethanol (95) and practically insoluble in ether.
Secobarbital Sodium is hygroscopic.
The solution of Secobarbital Sodium degrades on standing and its degradation is accelerated by heating.

Identification (1) Determine the infrared spectra of chloroform solutions of the residue obtained from the Assay and Secobarbital RS as directed in the solution method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Ignite about 0.5 g: the residue effervesces with acids and responds to the Qualitative Tests for sodium.

pH The pH of a solution of Secobarbital Sodium (1 in 10) is between 9.7 and 10.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Secobarbital Sodium in 10 mL of a freshly boiled and cooled water: the solution is clear and has no undissolved material after 1 minute.

(2) ***Heavy metals***—Proceed with 1.0 g of Secobarbital Sodium according to Method 2 and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

(3) ***Isomer content***—Weigh accurately about 0.3 g of Secobarbital Sodium, dissolve in 5 mL of sodium hydroxide solution (1 in 100), add 0.3 g of a solution of 4-nitrobromobenzyl in 10 mL of ethanol (95), reflux for 30 minutes, cool, collect the precipitation on a small filter and wash with water, recrystallize the precipitate from 25 mL of ethanol (95) and dry at 105 °C for 30 minutes: the precipitate melts between 156 °C and 161 °C.

Bacterial Endotoxins Less than 0.9 EU/mg of secobarbital sodium, when Secobarbital Sodium is used a non-oral preparation without a further procedure for the removal of bacterial endotoxins.

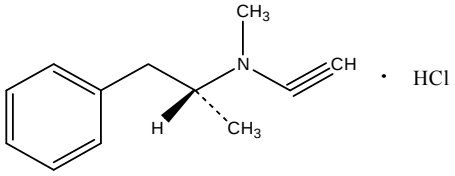
Loss on Drying Not more than 3.0 % (1 g, 80 °C, 5 hours).

Assay Dissolve about 0.5 g of Secobarbital Sodium, accurately weighed, in 15 mL of water in a separatory funnel. To this solution, add 2 mL of hydrochloric acid, shake and extract the liberated Secobarbital with eight times 25 mL volumes of chloroform. Test for completeness of extraction by extracting with an additional 10 mL of chloroform and evaporating the solvent: not more than 0.5 mg of residue remains. Filter the extracts into a tared beaker and finally rinse the separatory funnel and the filter with several small volumes of chloroform. Evaporate the combined filtrate and washings in a steam-bath with the aid of a current of air just to dryness. Dissolve the residue in 2 mL of ethanol (99.5) and evaporate to dryness. Repeat the dissolution and evaporation with 2 mL of ethanol (99.5), dry the residue at 100 °C for 2 hours. and weigh its mass after cooling.

Amount (mg) of secobarbital sodium ($C_{12}H_{17}N_2NaO_3$)
= Amount (mg) of the residue × 1.092

Containers and Storage ***Containers***—Tight containers.

Selegiline Hydrochloride



$C_{13}H_{17}N \cdot HCl$: 223.74

(2*R*)-*N*-Methyl-*N*-prop-2-yn-1-yl-1-phenylpropan-2-amine hydrochloride [*14611-52-0*]

Selegiline Hydrochloride contains not less than 98.0 % and not more than 101.0 % of selegiline hydrochloride ($C_{13}H_{17}N \cdot HCl$), calculated on the dried basis.

Description Selegiline Hydrochloride is a white crystalline powder and is ordorless.
Selegiline Hydrochloride is freely soluble in water, in chloroform, or in methanol.

Identification (1) Determine the absorption spectra of the solutions of Selegiline Hydrochloride and Selegiline Hydrochloride RS in water (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry:

both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Selegiline Hydrochloride and Selegiline Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) The retention time of the pricipal peak from the test solution corresponds to that from the standard solution, as obtained in the Assay.

(4) The solution of Selegiline Hydrochloride in water (1 in 50) responds to the Qualitative Tests for chloride (the Flame Coloration Test 2).

Specific Optical Rotation $[\alpha]_D^{20}$: −10 ~ −12° (2.0 g, water, 20 mL, 100 mm).

Melting Point 141 ~ 145 °C.

Purity (1) ***Heavy metals*—**Proceed with 1.0 g of Selegiline Hydrochloride**,** according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (20 ppm).

(2) ***Related substances*—**Dissolve about 50 mg of Selegiline Hydrochloride, in the mobile phase to make exactly 50 mL, and use this solution as the test solution. Separately, dilute 10.0 mL of the system suitability solution with the mobile phase to make exactly 100 mL. Add to 10.0 mL of this solution the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography, determine all peak areas by the automatic integration method, and calculate the percentage of each related substance in the portion of Selegiline Hydrochloride. Not more than 0.2 % of any individual related substance is found, and the sum of all related substances is not more than 1.0 %.

$$\text{Amount (\%) of each related substance} = 5000 \times \frac{C}{W} \times \frac{A_i}{A_S}$$

C: Concentration (mg/mL) of Selegiline Hydrochloride in the standard solution.

W: Amount (mg) of Selegiline Hydrochloride taken to prepare the test solution.

A_i: Peak area for each related substance from the test solution.

A_S: Peak area of selegiline from the standard solution.

Operating conditions

Detector, column, mobile phase, flow rate, and system suitability solution: Proceed as directed in the Assay.

System suitability

System performance: When the test is performed with 20 μL of the standard solution according to the above operating conditions, the resolution between the peaks of methamphetamine and selegiline is not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of selegiline is not more than 5.0 %.

Time span of measurement: About 3 times as long as the retention time of selegiline.

Loss on Drying Not more than 1.0 % (1 g, 60 °C, vacuum, 3 hours).

Residue on Ignition Not more than 0.2 % (1 g).

Assay Dissolve about 50 mg each of Selegiline Hydrochloride and Selegiline Hydrochloride RS, accurately weighed, in the mobile phase to make 50 mL. Add to 10.0 mL each of the solutions the mobile phase to make 100 mL, and use these solutions as the test solution and the standard solution. Perform the test with 20 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of selegiline hydrochloride.

$$\text{Amount (mg) of selelgiline hydrochloride } (C_{13}H_{17}N\cdot HCl) = \text{Amount (mg) of Selelgiline Hydrochloride} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 205 nm).

Column: A stainless steel column about 3.9 mm internal diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 to 10 μm in particle diameter).

Mobile phase: a mixture of phosphate buffer solution and acetonitrile (80:20).

Flow rate: 1 mL/min.

System suitability

System performance: Dissolve 10 mg of Methamphetamine Hydrochloride RS and 10 mg of Selegiline Hydrochloride RS in the mobile phase to obtain 100 mL, and use this solution as the system suitability solution. When the procedure is run with 20 μL of this solution as directed in the Liquid chromatography under the above operating conditions, the resolution between the peaks of methamphetamine and selegiline is not less than 3.

System repeatability: When the test is repeated 5 times with 20 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the peak area of selegiline is not more than 2.0 %.

Phosphate buffer solution—Prepare a solution of

0.1 mol/L monobasic ammonium phosphate, and adjust with phosphoric acid to a pH of 3.1.

Containers and Storage ***Containers*****—**Tight containers.

Storage**—**Light-resistant.

Selenium Sulfide

SeS_2: 143.09

[*7488-56-4*]

Selenium Sulfide contains not less than 52.0 % and not more than 55.5 % of selenium (Se: 78.96).

Description Selenium Sulfide appears as red-brown to orange powder and has a slight odor.
Selenium Sulfide is practically insoluble in water or in organic solvents.

Identification (1) Filter 20 mL of the solution of Selenium Sulfide prepared as directed in the Assay and to 10 mL of the filtrate, add 5 mL of water and 5 g of urea. Heat to boiling, cool and add 2 mL of potassium iodide solution (1 in 10): an orange color is observed and it darkens rapidly.

(2) Allow the solution obtained in Identification (1) to stand for 10 minutes, filter and to the filtrate, add 10 mL of barium chloride TS: the solution becomes turbid.

Purity ***Soluble selenium compounds***—Mix 10.0 g of Selenium Sulfide with 100 mL of water in a 250 mL flask, allow to stand for 1 hour, with frequent shaking and filter. Pipet 10.0 mL of the filtrate, add 2 mL of 2.5 mol/L formic acid, add 50 mL of water, mix and adjust, if necessary, to a pH of 2.5 ± 0.5. Add 2 mL of freshly prepared 3,3'-diamino-benzidine hydrochloride solution (1 in 200), mix, allow to stand for 45 minutes and adjust with ammonium hydroxide to a pH of 6.5 ± 0.5. Transfer to a separatory funnel, add 10.0 mL of toluene, shake vigorously for 1 minute, allow the layer to separate, discard the aqueous phase and use the toluene layer as the test solution. Pipet 50 mL of the standard selenium stock solution, add water to make exactly 100 mL. Pipet 10 mL of this solution, add 2 mL of 2.5 mol/L of formic acid and prepare a solution as directed in the same manner as the test solution and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry. Determine the absorbances of the test solution and the standard solution at 420 nm using a blank consisting of the same quantities of the same reagents treated in the same manner as the test solution: the absorbance of the test solution is not greater than that of the standard solution (not more than 5 ppm).

Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 0.1 g of Selenium Sulfide, add 25 mL of fuming nitric acid and digest over gentle heat until no further dissolution occurs. Cool, transfer the solution to a volumetric flask containing 100 mL of water, cool again and add water to make exactly 250 mL and mix. Pipet 50 mL of the solution into a flask, add 25 mL of water and 10 g of urea and heat to boiling. Cool, add 3 mL of starch TS, then add 10 mL of potassium iodide solution (1 in 10) and immediately titrate with 0.05 mol/L sodium thiosulfate VS. Perform a blank determination and make any necessary correction.

Each mL of 0.05 mol/L sodium thiosulfate VS
= 0.987 mg of Se

Containers and Storage ***Containers***—Well-closed containers.

Silver Nitrate

$AgNO_3$: 169.87

[*7761-88-8*]

Silver Nitrate, when dried, contains not less than 99.8 % and not more than 101.0 % of silver nitrate ($AgNO_3$).

Description Silver Nitrate appears as lustrous, colorless or white crystals.
Silver Nitrate is very soluble in water, soluble in ethanol (95) and practically insoluble in ether.
Silver Nitrate gradually turns to grayish black by light.

Identification A solution of Silver Nitrate (1 in 50) responds to the Qualitative Tests for silver salt and for nitrate.

Purity (1) ***Clarity and color of solution and acidity or alkalinity***—Dissolve 1.0 g of Silver Nitrate in 10 mL of a freshly boiled and cooled water: the solution is clear, colorless and neutral.

(2) ***Bismuth, copper and lead***—To 5 mL of a solution of Silver Nitrate (1 in 10), add 3 mL of ammonia TS: the solution is clear and colorless.

Loss on Drying Not more than 0.2 % (2 g, silica gel, light resistant, 4 hours).

Assay Weigh accurately 0.7 g of Silver Nitrate, previously powdered and dried, dissolve in 50 mL of water, add 2 mL of nitric acid and titrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS).

Each mL of 0.1 mol/L ammonium thiocyanate VS
= 16.987 mg of $AgNO_3$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Silver Protein

Silver Protein is a compound of silver and proteins. Silver Protein contains not less than 7.5 % and not more than 8.5 % of silver (Ag: 107.87).

Description Silver Protein is a pale yellow-brown to brown powder and is odorless.
1 g of Silver Protein dissolves slowly in 2 mL of water and Silver Protein is practically insoluble in ethanol (95), in ether or in chloroform.

pH—A solution of 1.0 g of Silver Protein in 10 mL of water is between 7.0 and 8.5.
Silver Protein is slightly hygroscopic.
Silver Protein is affected by light.

Identification (1) To 10 mL of a solution of Silver Protein (1 in 100), add 2 mL of dilute hydrochloric acid, shake frequently for 5 minutes and filter. To the filtrate, add 5 mL of a solution of sodium hydroxide (1 in 10) and add 2 mL of diluted copper (II) sulfate TS (2 in 25): a purple color is observed.

(2) To 5 mL of a solution of Silver Protein (1 in 100), add drop-wise iron (III) chloride TS: the color of the solution fades and a precipitate is gradually formed.

(3) Incinerate 0.2 g of Silver Protein by ignition, dissolve the residue in 1 mL of nitric acid by warming and add 10 mL of water: this solution responds to the Qualitative Tests (1) for silver salt.

Purity ***Silver salt***—Dissolve 0.10 g of Silver Protein in 10 mL of water and filter. To the filtrate, add 1 mL of potassium chromate TS: no turbidity is produced.

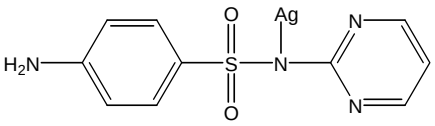
Assay Transfer about 1 g of Silver Protein, accurately weighed, to a decomposition flask, add 10 mL of sulfuric acid, cover the flask with a funnel and boil for 5 minutes. Cool, add drop-wise 3 mL of nitric acid with caution and heat for 30 minutes without boiling. Cool, add 1 mL of nitric acid, boil and, if necessary, repeat this operation until the solution becomes colorless at cooling. After cooling, transfer the solution to a 250 mL of Erlenmeyer flask, wash the decomposition flask with 100 mL of water, combine the washings to the Erlenmeyer flask and titrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 3 mL of ammonium iron (III) sulfate TS).

Each mL of 0.1 mol/L ammonium thiocyanate VS
= 10.787 mg of Ag

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Silver Sulfadiazine



$C_{10}H_9AgN_4O_2S$: 357.14

Silver *N*-(4-aminobenzene)sulfonyl-*N*′-(pyrimidin-2-yl)azanide [*22199-08-2*]

Silver Sulfadiazine, when dried, contains not less than 99.0 % and not more than 102.0 % of silver sulfadiazine ($C_{10}H_9AgN_4O_2S$).

Description Silver Sulfadiazine is a white to pale yellow, crystalline powder and is odorless.
Silver Sulfadiazine is practically insoluble in water, in ethanol (95) or in ether.
Silver Sulfadiazine dissolves in ammonia TS.
Silver Sulfadiazine is gradually colored by light.

Melting point—About 275 °C (with decomposition).

Identification Determine the infrared spectra of Silver Sulfadiazine and Silver Sulfadiazine RS, previously dried, as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity (1) ***Nitrate***—To 250 mL of water, add 1.0 g of Silver Sulfadiazine, shake well for 50 minutes, filter and use this filtrate as the test solution. Separately, weigh accurately 0.25 g of potassium nitrate and dissolve in water to make exactly 2000 mL. Pipet 5.0 mL of this solution and add water to make exactly 200 mL and use this solution as the standard solution. Pipet 2.0 mL each of the test solution and the standard solution and add 5 mL of a solution of chromotropic acid in sulfuric acid (1 in 10000) and sulfuric acid to make exactly 10 mL. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 408 nm as directed under Ultraviolet-visible Spectrophotometry, using a solution prepared with exactly 2.0 mL of water in the same manner as the blank: A_T is not larger than A_S (not more than 0.05 %).

(2) ***Related substances***—Dissolve 50 mg of Silver Sulfadiazine in 5 mL of a mixture of ethanol (95) and ammonia solution (28) (3 : 2) and use this solution as the test solution. Pipet 2.0 mL of the test solution and add a mixture of ethanol (95) and ammonia solution (28) (3 : 2) to make exactly 20 mL. Pipet 2.0 mL of this solution, add a mixture of ethanol (95) and ammonia solution (28) (3 : 2) to make exactly 20 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate

of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28) (10 : 5 : 2) to a distance of about 15 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and spot of the starting point from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, P_2O_5, 80 °C, 4 hours).

Residue on Ignition 41.0 ~ 45.0 % (1 g).

Silver Content Weigh accurately 50 mg of Silver Sulfadiazine, previously dried, dissolve in 2 mL of nitric acid and add water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL and use this solution as the test solution. Separately, measure accurately a suitable portion of standard silver solution for Atomic Absorption Spectrophotometry, dilute with water to make a solution containing 1.0 to 2.0 μg of silver (Ag: 107.87) per mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Atomic Absorption Spectrophotometry according to the following conditions and calculate the silver content of the test solution from the calibration curve obtained from the absorbance of the standard solution; content of silver is not less than 28.7 % and not more than 30.8 %.

Gas: Dissolved acetylene – Air.
Lamp: A silver hollow cathode lamp.
Wavelength: 328.1 nm.

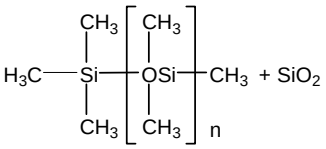
Assay Weigh accurately about 0.1 g each of Silver Sulfadiazine and Silver Sulfadiazine RS, each previously dried and add ammonia TS to make exactly 100 mL. Pipet 1 mL each of these solutions, add water to make exactly 100 mL and use these solutions as the test solution and the standard solution, respectively. Determine the absorbances, A_T and A_S, of these solutions at 255 nm, using a solution, prepared with exact 1 mL of ammonia TS and a sufficient water to make exactly 100 mL, as the blank.

$$\text{Amount (mg) of silver sulfadiazine } (C_{10}H_9AgN_4O_2S) = \text{Amount (mg) of Silver Sulfadiazine RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Simethicone



Poly(dimethylsiloxane), silicon dioxide [*8050-81-5*]

Simethicone is a mixture of fullyl methylated linear siloxane polymers containing repeating units of the formula $[-(CH_3)_2SiO-]_n$, stabilized with trimethylsiloxy endblocking units of the formular $[(CH_3)_2SiO-]$ and silicon dioxide. Simethicone contains not less than 90.5 % and not more than 99.0 % of polydimethylsiloxane ($[(CH_3)_2SiO-]_n$) and not less than 4.0 % and not more than 7.0 % of silicone dioxide.

Description Simethicone is a translucent, gray, viscous liquid.
Simethicone is insoluble in water or ethanol (95). The liquid phase of Simethicone is soluble in chloroform, in ether or in benzene, but silicon dioxide remains as residue in these solvents.

Identification (1) Determine the infrared spectra of the test solution and the standard solution in the Assay as directed in the solution method under Infrared Spectrophotometry using 0.5 mm cell: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity ***Heavy metals***—Weigh 1.0 g of Simethicone, dissolve in 10 mL of chloroform, mix and add chloroform more to make 20 mL. Add 1.0 mL of a freshly prepared 0.002 % solution of dithizone chloroform, 0.5 mL of water and 9.5 mL of a mixture of ammonia TS and 0.2 % solution of hydroxylamine hydrochloride (1 : 9) and use this solution as the test solution. Separately, to 20 mL of chloroform, add 1.0 mL of a freshly prepared 0.002 % solution of dithizone in chloroform, 0.5 mL of standard lead solution and 0.5 mL of a mixture of ammonia TS and 0.2 % solution of hydroxylamine hydrochloride (1 : 9) and use this solution as the standard solution. Immediately shake both solutions vigorously for 1 minute: Any red color in the test solution is not more intense than that in the standard solution (Not more than 5 ppm).

Loss on Drying Not more than 18.0 % (15.0 g, 200 °C, 4 hours)

Defoaming Activity Dissolve 1.0 g of octoxynol in 100 mL of water and use this solution as the foaming solution. Weigh accurately 0.2 g of Simethicone and transfer to a 60 mL bottle. Add 50 mL of *t*-butyl alcohol, cap the bottle, shake and use this solution as the test solution. Warm this solution slightly, if necessary. Add, dropwise, 0.5 mL of the test solution to a clean,

cylindrical 250 mL glass jar, fitted with a 50 mm cap, containing 100 mL of the foaming solution. Cap the jar and clamp it in an upright position on a wrist-action shaker. Employing a radius of 13.3 ± 0.4 cm measured from center of shaft to center of bottle, shake for 15 seconds through an arc of 10° at a frequency of 300 ± 30 strokes per minute. Record the time required for the foam to collapse. The time for foam collapse is determined at the instant the first portion of foam-free liquid surface appears, measured from the end of the shaking period. The defoaming activity time does not exceed 15 minutes.

Content of Silicon Dioxide Transfer 3.00 g each of Simethicone, Simethicone RS and dimethicone (500 mm^2/s) to a screw-capped bottle, add 10.0 mL of n-hexane, cap, mix by shaking and use these solutions as the test solution, the standard solution and dimethicone solution respectively. Perform the test as directed in the solution method under Infrared Spectrophotometry with these solutions. Using 0.1 mm fixed cells, determine the absorbance spectra of these solutions between 7 and 9 μm, using *n*-hexane as the blank. Determine the absorbance of the test solution, the standard solution and the dimethicone solution at the wavelength of minimum absorbance at about 8.2 μm observed in the spectrum obtained from the dimethicone solution.

$$\text{Amount (\%) of silicon dioxide } (SiO_2) \text{ in Simethicone} = \text{Amount (\%) of silicon dioxide in Simethicone RS} \times \frac{A_T - A_D}{A_S - A_D}$$

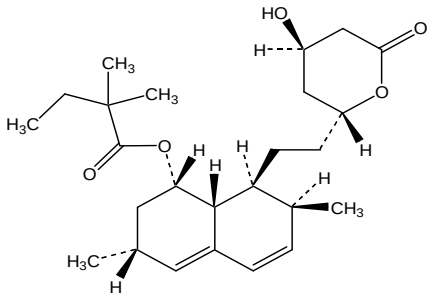
A_D: Absorbance of dimethicone solution
A_T: Absorbance of the test solution
A_S: Absorbance of the standard solution

Assay Transfer about 50 mg each of Simethicone and polydimethylsiloxane RS, accurately weighed, to a round, narrow-mouth, screw-capped, 120-mL bottle, add 25.0 mL of toluene and swirl to disperse. Add 50 mL of diluted hydrochloric acid (2 in 5), close the bottle securely with a cap having an inert liner and shake for 5 minutes, accurately timed, on a reciprocating shaker at a frequency of about 200 oscillations per minute and a stroke of 38 ± 2 mm. Transfer the mixture to a 125-mL separatory funnel and remove about 5 mL of the toluene layer to a 15-mL screw-capped test tube containing 0.5 g of anhydrous sodium sulfate. Close the tube with a screw-cap having an inert liner, agitate vigorously, centrifuge the mixture until a clear supernatant liquid is obtained and use these solutions as the test solution and the standard solution, respectively. Perform the test as directed in the solution method under Infrared Spectrophotometry with these solutions, using a solution, prepared with 25.0 mL of toluene in the same manner to the test solution, as the blank. Determine the absorbances, A_T and A_S, of these solutions in 0.5-mm cells at the wavelength of maximum absorbance at about 7.9 μm, with an Infrared Spectrophotometer, respectively.

$$\text{Amount (mg) of polydimethylsiloxane } ([-(CH_3)_2SiO-]_n) \text{ in Simethicone} = \text{Concentration (mg/mL) of Polydimethylsiloxane RS} \times \frac{A_T}{A_S} \times 25$$

Containers and Storage ***Containers***—Tight containers.

Simvastatin



$C_{25}H_{38}O_5$: 418.57

[(1*S*,3*R*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-Hydroxy-6-oxooxan-2-yl]ethyl]-3,7-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl] 2,2-dimethylbutanoate [*79902-63-9*]

Simvastatin contains not less than 98.0 % and not more than 101.0 % of simvastatin ($C_{25}H_{38}O_5$), calculated on the dried basis. Simvastatin may contain a suitable antioxidant.

Description Simvastatin appears as white powder. Simvastatin is freely soluble in methanol, in ethanol (95), or in chloroform, sparingly soluble in propylene glycol, very slightly soluble in hexane, and practically insoluble in water.

Identification (1) Weigh 10 mg each of Simvastatin and Simvastatin RS, dissolve in acetonitrile to make 100 mL, pipet 5.0 mL each of these solutions, add separately acetonitrile to make 50 mL. Determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Simvastatin and Simvastatin RS, previously dried, as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +285 ~ 298° (after drying, 0.10 g, acetonitrile, 20 mL, 100 mm).

Purity (1) ***Clarity of solution***—Dissolve 1.0 g of Simvastatin in 10 mL of methanol: the solution is clear and transparent. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry: the absorbance at 440 nm is not more than 0.10.

(2) ***Heavy metals***—Proceed with 1.0 g of Simvastatin according to Method 2. and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Weigh accurately about 30 mg of Simvastatin, dissolve in a mixture of acetonitrile and 0.01 mol/L potassium dihydrogen phosphate TS (pH 4.0) (3 : 2) to make exactly 20 mL, and use this solution as the test solution. Perform the test with 5 μL of the test solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of the test solution by the automatic integration method. Calculate the amount of each peak by the area percentage method: the amounts of the related substance, having the relative retention times of 0.45, 0.80, 2.42, and 3.80 with respect to simvastatin, is not more than 0.2 %, respectively, the related substance having the relative retention time of 2.38 is not more than 0.3 %, the related substance having the relative retention time of 0.60 is not more than 0.4 %, and each related substance other than simvastatin and other than those mentioned above is not more than 0.1 %. The total amount of the peaks other than simvastatin and other than the related substance having the relative retention time of about 0.60 with respect to simvastatin is not more than 1.0 %.

Operating conditions

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: Control the gradient by mixing mobile phases A and B as directed in the following table.

Mobile phase A: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (1 : 1)

Mobile phase B: A solution of phosphoric acid in acetonitrile (1 in 1000)

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-4.5	100	0
4.5-4.6	100→95	0→5
4.6-8.0	95→25	5→75
8.0-11.5	25	75

Flow rate: 3.0 mL/minute

System suitability

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Pipet 0.5 mL of the test solution, add a mixture of acetonitrile and 0.01 mol/L potassium dihydrogen phosphate TS (pH 4.0) (3 : 2) to make exactly 100 mL, and use this solution as the system suitability solution. Pipet 2 mL of the system suitability solution, and add a mixture of acetonitrile and 0.01 mol/L potassium dihydrogen phosphate TS (pH 4.0) (3 : 2) to make exactly 10 mL. Confirm that the peak area of simvastatin obtained from 5 μL of this solution is equivalent to 16 to 24 % of that from the system suitability solution.

System repeatability: When the test is repeated 6 times with 5 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the peak areas of simvastatin is not more than 1.0 %.

Time span of measurement: About 5 times as long as the retention time of simvastatin.

(4) ***Lovastatin***—Calculate the amount (%) of lovastatin from the chromatogram of the test solution and the standard solution, A_T and A_S, respectively, as directed under the Assay, the percentage of lovastatin is not greater than 1.0 %.

$$\text{Amount (\%) of lovastatin} = 10000 \times \frac{C}{W} \times \frac{A_T}{A_S}$$

C: Concentration (mg/mL) of Lovastatin RS in the standard solution

W: Amount (mg) of Simvastatin

Loss on Drying Not more than 0.5 % (1.0 g, in vacuum, 60 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1.0 g).

Assay Weigh accurately about 30 mg each of Simvastatin and Simvastatin RS (previously determine the loss on drying in the same manner as Simvastatin), dissolve each in a mixture of acetonitrile and 0.01 mol/L potassium dihydrogen phosphate (pH 4.0) (3 : 2) to make exactly 20 mL, and use these solutions as the test solution and standard solution. Perform the test as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of simvastatin in each solution.

$$\text{Amount (mg) of Simvastatin } (C_{25}H_{38}O_5) = W_S \times \frac{A_T}{A_S}$$

W_S: Amount (mg) of Simvastatin RS, calculated on the dried basis

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 238 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 33 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: A mixture of diluted phosphoric acid

(1 in 1000) and acetonitrile (1 : 1).

Flow rate: Adjust the flow rate so that the retention time of simvastatin is about 3 minutes.

System suitability

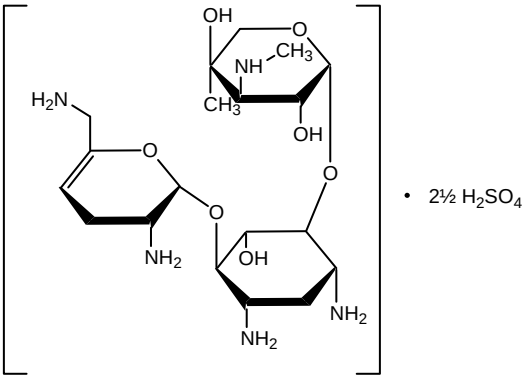
System performance: Dissolve 3 mg of lovastatin in 2 mL of the standard solution. When the procedure is run with 5 μL of this solution under the above operating conditions, lovastatin and simvastatin are eluted in this order with the resolution between these peaks being not less than 3.0.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of simvastatin is not more than 1.0 %.

Containers and Storage ***Containers***—Well-closed containers

Storage—Under nitrogen atmosphere.

Sisomicin Sulfate



$C_{19}H_{37}N_5O_7 \cdot 2½H_2SO_4$: 692.72

(2*R*,3*R*,4*R*,5*R*)-2-{[(1*S*,2*S*,3*R*,4*S*,6*R*)-4,6-Diamino-3-{[(2S,3R)-3-amino-6-(aminomethyl)-3,4-dihydro-2*H*-pyran-2-yl]oxy}-2-hydroxycyclohexyl]oxy}-5-methyl-4-(methylamino)oxane-3,5-diol pentahemisulfate [*53179-09-2*]

Sisomicin Sulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of *Micromonospora inyoensis*.

Sisomicin Sulfate contains not less than 590 μg (potency) and not more than 700 μg (potency) per mg of sisomicin ($C_{19}H_{37}N_5O_7$: 447.53), calculated on the dried basis.

Description Sisomicin Sulfate appears as white to pale yellowish white powder.

Sisomicin Sulfate is very soluble in water, and practically insoluble in ethanol (95).

Sisomicin Sulfate is hygroscopic.

Identification (1) Dissolve 50 mg of Sisomicin Sulfate in 5 mL of water, and add 0.3 mL of bromine TS: the color of the solution disappears immediately.

(2) Dissolve separately 15 mg (potency) each of Sisomicin Sulfate and Sisomicin Sulfate RS in 5 mL of water, and use these solutions as the test solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, chloroform, ammonia solution (28), and acetone (2 : 2 : 1 : 1) to a distance of about 15 cm, and air-dry the plate. Spray evenly 0.2 % ninhydrin-water saturated 1-butanol TS on the plate, and heat at 100 °C for about 5 minutes: the principal spots from the test solution and standard solution show a red-purple to red-brown color and the same R_f value.

(3) Sisomicin Sulfate responds to the Qualitative Tests for sulfate.

Specific Optical Rotation $[\alpha]_D^{20}$: +100 ~ +110° (0.25 g calculated on the dried basis, water, 25 mL, 100 mm)

pH The pH of a solution obtained by dissolving 1 g (potency) of Sisomicin Sulfate in 10 mL of water is between 3.5 and 5.5.

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Sisomicin Sulfate in 5 mL of water: the solution is clear and colorless to pale yellow.

(2) ***Heavy metals***—Proceed with 1.0 g of Sisomicin Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Dissolve an amount of Sisomicin Sulfate, equivalent to 50 mg calculated on the dried basis, in 10 mL of water, and use this solution as the test solution. Pipet 0.5 mL, 1 mL, and 1.5 mL of the test solution, add water to make exactly 50 mL each, and use these solutions as standard solutions (1), (2), and (3). Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and standard solutions (1), (2), and (3) on a plate of silica gel for thin-layer chromatography. Develp the plate with a mixture of methanol, chloroform, ammonia solution (28), and acetone (2 : 2 : 1 : 1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2 % ninhydrin-water saturated 1-butanol TS on the plate, and heat at about 100 °C for about 5 minutes. Calculate the amount of the spots other than the principal spot from the test solution by comparing with each of the principal spots from standard solutions (1), (2), and (3): the spots having the R_f values of about 0.35 and 0.30 are not more intense than the spot from standard solution (3), respectively, the spot of galamine having the R_f value of about 0.25 is not more intense than the spot from standard solution

(1), and the total amount of related substances is not more than 6 %.

Loss on Drying Not more than 15.0 % (0.15 g, reduced pressure not exceeding 0.67 kPa, 110 °C, 3 hours). Avoid moisture absorption when taking the sample.

Residue on Ignition Not more than 1.0 % (1 g)

Sterility Test It meets the requirement, when Sisomicin Sulfate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.50 EU/mg (potency) of sisomicin, when Sisomicin Sulfate is used in a sterile preparation.

Assay ***The Cylinder-plate method*** (1) Agar media for seed and base layer- Use the culture medium in I 2 1) (3) ① under Microbial Assay for Antibiotics. Adjust the pH of the medium so that it will be between 7.8 and 8.0.

(2) Agar medium for transferring test organisms- Use the culture medium in I 2 1) (3) ① under Microbial Assay for antibiotics.

(3) Test organism- *Staphylococcus epidermidis* ATCC 12228

(4) Weigh accurately about 25 mg of Sisomicin Sulfate, dissolve in 25 mL of 0.1 mol/L phosphate buffer solution (pH 8.0) so that each mL contains 1 mg (potency), and use this solution as the test stock solution. Pipet a suitable amount of the test stock solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) so that each mL contains 1.00 μg (potency) and 0.25 μg (potency), and use these solutions as the high concentration test solution and low concentration test solution, respectively. Separately, weigh accurately a suitable amount of Sisomicin Sulfate RS, dissolve in 0.1 mol/L phosphate buffer solution (pH 8.0) so that each mL contains 1 mg (potency), and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 5 °C, and use within 7 days. Pipet a suitable amount of the standard stock solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) so that each mL contains 1.00 μg (potency) and 0.25 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively. Perform the test with these solutions as directed in I 8 under Microbial Assay for Antibiotics.

Containers and Storage ***Containers*****—**Tight containers.

Storage**—**Light-resistant, under nitrogen or argon atmosphere, and at a temperature not exceeding -20 °C.

Sisomicin Sulfate Injection

Sisomicin Sulfate Injection contains not less than 90.0 % and not more than 120.0 % of the labeled amount of sisomicin ($C_{19}H_{37}N_5O_7$: 447.53).

Method of Preparation Prepare as directed under Injections, with Sisomicin Sulfate.

Description Sisomicin Sulfate Injection appears as clear and colorless to pale yellow liquid.

Identification Perform the test as directed in the Identification (2) under Sisomicin Sulfate. Use the undiluted stock solution as the test solution.

pH 2.5 ~ 5.5.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.50 EU/mg (potency) of sisomicin.

Foreign Insoluble Matter Test It meets the requirement.

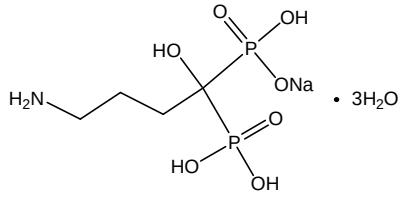
Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay ***The Cylinder-plate method*** Proceed as directed in the Assay under Sisomicin Sulfate. Pipet a suitable amount of Sisomicin Sulfate Injection according to the labeled potency, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make a solution of suitable concentration, pipet a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make the concentration of (3), and use this solution as the test solution.

Containers and Storage ***Containers*****—**Hermetic containers.

Sodium Alendronate Hydrate



$C_4H_{12}NNaO_7P_2 \cdot 3H_2O$: 325.12

Sodium hydrogen (4-amino-1-hydroxy-1-phosphonobutyl)phosphinate trihydrate [*121268-17-5*]

Sodium Alendronate Hydrate contains not less than 98.0 % and not more than 102.0 % of sodium alendronate ($C_4H_{12}NNaO_7P_2$: 257.07), calculated on the dried basis.

Description Sodium Alendronate Hydrate is a white crystalline powder.
Sodium Alendronate Hydrate is soluble in water, very slightly soluble in methanol, and practically insoluble in dichloromethane.

Identification (1) Determine the infrared spectra of Sodium Alendronate Hydrate and Sodium Alendronate Hydrate RS, previously dried, as directed in the paste method Under the Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Sodium Alendronate Hydrate responds to the Qualitative Tests for (1) sodium.

pH Dissolve 0.5 g of Sodium Alendronate Hydrate in 50 mL of water: the pH of this solution is between 4.0 and 5.0.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Sodium Alendronate Hydrate according to Method 2. and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Weigh accurately about 30 mg of Sodium Alendronate Hydrate, dissolve in 2.94 % solution of sodium citrate to make exactly 50 mL. Pipet 5.0 mL of this solution, transfer into the 50 mL polypropylene tube with cap for centrifuge containing 5.0 mL of 1.91 % solution of sodium borate, add 5 mL of acetonitrile and 5 mL of 0.4 % 9-fluorenylmethylchloroformate in acetonitrile, prepared before use, shake for 45 seconds, allow to stand for 30 minutes. Add 20 mL of the dichloromethane, shake for 1 minute vigorously, and centrifuge for 5 to 10 minutes. Use the clear upper water layer as the test solution. Separately, prepare for the blank solution by the same procedure as for the test solution with 5.0 mL of 2.94 % solution of sodium citrate. Perform the test with exactly 20 μL each of the test solution and the blank solution as directed under Liquid Chromatography according to the following conditions: Discard the peak being the same retention time as the peak from blank solution. In the determination of the each amount (%) of related substances each amount of related substances is not more than 0.1 % and total amount of related substances is not more than 0.5 %.

$$\text{Amount (\%) of each related substance} = 100 \times \frac{A_i}{A_S}$$

A_i: Peak area of the related substance
A_S: Total area of all related substance

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 266 nm).

Column: A stainless steel column about 4.1 mm in internal diameter and about 25 cm in length, packed with spherical styrene-divinylbenzene polymer for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 45 ºC.

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: the mixture of buffer solution and acetonitrile (17 : 3)

Mobile phase B the mixture of acetonitrile and buffer solution (7 : 3)

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0	100	0
0-15	100→50	0→50
15-25	50→0	50→100
25-27	0→100	100→0
27-32	100	0

Flow rate: 1.8 mL/minute
System suitability

System performance: Weigh 60.0 mg of Sodium Alendronate Hydrate RS, dissolve in 2.94 % solution of sodium citrate to make 100 mL and use this solution as the stock solution. Proceed with 5.0 mL of the stock solution as directed above for the preparation of the test solution and use this solution as the standard solution. Separately, pipet 0.1 mL of the stock solution, add 2.94 % solution of sodium citrate to make exactly 100 mL, pipet 5.0 mL of this solution, proceed as directed above for the preparation of the test solution, and use this solution as the dilute standard solution. When the procedure is run with 20 μL of the standard solution and the dilute standard solution under the above operating condition, the symmetry factor of the principal peak with the standard solution is not more than 2.0 and signal-to-noise ratio of the peak, detected in the same location as the dilute standard solution, is not less than 3.

Buffer solution—Dissolve 5.88 g of sodium citrate dihydrate and 2.84 g of anhydrous disodium hydrogen phosphate in water to make 2000 mL. Adjust with phosphoric acid
to pH 8.

Loss on Drying 16.1 ~ 17.1 % (1 g, in vacuum, 145 °C, constant mass).

Assay Weigh accurately about 25 mg of Sodium Alendronate Hydrate, dissolve in 2.94 % solution of sodium citrate to make exactly 250 mL. Pipet 5.0 mL of this solution, transfer into the 50 mL polypropylene tube with cap for centrifuge containing 5 mL of the

1.91 % solution of sodium borate, add 5 mL of 0.05 % 9-fluorenyl-methylchloroformate in acetonitrile, prepared before use, shake for 30 seconds, allow to stand for 25 minutes at room temperature. Add 25 mL of the dichloromethane, shake for 1 minute vigorously, and centrifuge for 5 to 10 minutes. Use the clear upper water layer as the test solution. Separately, weigh accurately about 10 mg of Sodium Alendronate Hydrate RS, dissolve in 2.94 % solution of sodium citrate to make exactly 100 mL, and use this solution as the stock solution. Proceed with 5.0 mL of the stock solution as directed above for the preparation of the test solution, and use this solution as the standard solution. Also proceed with 5.0 mL of 2.94 % solution of sodium citrate as directed above for the preparation of the test solution, and use this solution as the blank solution. Perform the test with exactly 10 μL each of the blank solution, the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine principal peak area of the test and the standard solution, A_T and A_S, respectively.

$$\text{Amount (mg) of sodium alendronate}(C_4H_{12}NNaO_7P_2) = 250 \times C \times \frac{A_T}{A_S}$$

C: Concentration (mg/mL) of Sodium Alendronate RS in the standard solution, calculated on the anhydrous basis.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 266 nm).

Column: A stainless steel column about 4.1 mm in internal diameter and about 25 cm in length, packed with spherical styrene-divinylbenzene polymer for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 35 ºC.

Mobile phase: the mixture of buffer solution, acetonitrile and methanol (70 : 25 : 5)

Flow rate: 1.8 mL/minute

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating condition, the number of theoretical plates and the symmetry factor of the principal peak are not less than 1500 and not more than 1.5, respectively.

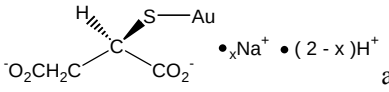
System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sodium alendronate is not more than 2.0 %.

Buffer solution—Dissolve 14.7 g of sodium citrate dihydrate and 7.05 g of anhydrous disodium hydrogen phosphate in water to make 1000 mL. Adjust with phosphoric acid to pH 8.

Containers and Storage ***Containers***—Well-closed containers.

Storage—At a temperature between 15 and 30 ºC.

Sodium Aurothiomalate



and enantiomer

Mixture of $C_4H_3AuNa_2O_4S$: 390.08 and $C_4H_4AuNaO_4S$: 368.10

Disodium; gold(1+); 2-sulfidobutanedioate
[*12244-57-4*]

Sodium Aurothiomalate contains not less than 49.0 % and not more than 52.5 % of gold (Au: 196.97), calculated on the anhydrous basis and corrected by the amount of ethanol

Description Sodium Aurothiomalate is a white to pale yellow powder or granule and is odorless.
Sodium Aurothiomalate is very soluble in water and practically insoluble in ethanol (95).
Sodium Aurothiomalate is hygroscopic.
Sodium Aurothiomalate changes in color by light to greenish pale yellow

Identification (1) To 2 mL of a solution of Sodium Aurothiomalate (1 in 10), add 1 mL of calcium nitrate tetrahydrate solution (1 in 10): a white precipitate is produced and it dissolves in dilute nitric acid and reappears on the addition of ammonium acetate TS.

(2) To 2 mL of a solution of Sodium Aurothiomalate (1 in 10), add 3 mL of silver nitrate TS: a yellow precipitate is produced and it dissolves in an excess of ammonia TS.

(3) Place 2 mL of a solution of Sodium Aurothiomalate (1 in 10) in a porcelain crucible, add 1 mL of ammonia TS and 1 mL of hydrogen peroxide solution (30), evaporate to dryness and ignite. Add 20 mL of water to the residue and filter: the residue on the filter paper occurs as a yellow or dark yellow powder or yellow or dark yellow granule.

(4) The filtrate obtained in (3) responds to the Qualitative Tests for sodium salt.

(5) The filtrate obtained in (3) responds to the Qualitative Tests for sulfate

pH Dissolve 1.0 g of Sodium Aurothiomalate in 10 mL of water: the pH of this solution is between 5.8 and 6.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Sodium Aurothiomalate in 10 mL of water: the solution is clear and pale yellow.

(2) ***Heavy metals***—Proceed with 1.0 g of Sodium Aurothiomalate according to Method 2 and perform the

test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Sodium Aurothiomalate according to Method 3 and perform the test (not more than 2 ppm).

(4) ***Ethanol***—Weigh accurately about 0.2 g of Sodium Aurothiomalate, add exactly 3 mL of the internal standard solution and 2 mL of water to dissolve, and use this solution as the test solution. Separately, pipet 3 mL of ethanol (99.5), and add water to make exactly 1000 mL. Pipet 2 mL of this solution, add exactly 3 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 2 μL each of the test solution and the standard solution as directed under Gas Chromatography according to the following conditions, and determine the ratios of the peak area of ethanol to that of the internal standard, Q_T and Q_S: the amount of ethanol is not more than 3.0 %

$$\text{Amount (mg) of ethanol} = Q_T / Q_S \times 6 \times 0.793$$

0.793: Density (g/mL) of ethanol at 20 °C

Internal standard solution—A solution of 2-propanol (1 in 500).

Operating conditions

Detector: Hydrogen flame-ionization detector.

Column: A column 3 mm in internal diameter and 3 m in length, packed with porous styrene–divinylbenzene copolymer for gas chromatography (150 to 180 μm in particle diameter) (average pore size: 0.0085 μm; 300-400 m^2/g).

Column temperature: A constant temperature of about 180 °C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 7 minutes.

Systemic suitability

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, ethanol and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability:When the test is repeated 6 times with 2 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ethanol to that of the internal standard is not more than 2.0 %.

(5) ***Glycerin***—This procedure is based on the absorption characteristics of a sodium-copper-glycerin complex. The stability of the complex is such that all measurements must be taken within 1 hour, and thoroughly rinse all glassware used in this procedure with water to avoid blank errors.

Weigh accurately about 400 mg of Sodium Aurothiomalate, transfer to a 10 mL volumetric flask, dissolve in 5.0 mL of water and use this solution as the test solution. Separately, pipet a portion of glycerin and dissolve in water to render the concentration of the solution to be exactly 8mg/mL. Take 1.0 mL, 2.0 mL and 3.0 mL of this solution, transfer each to a 10 mL volumetric flask, add 4.0 mL, 3.0 mL and 2.0 mL of water, respectively, and use these solutions as standard glycerin solutions (1), (2) and (3). Transfer 5.0 mL of water to a 10 mL volumetric flask and use this solution as the blank solution. Add 1.0 mL of sodium hydroxide solution to each of the standard glycerin solutions, blank solution and test solution and mix. Add copper chloride solution to each solution in 0.1 mL volumes and shake well, observing the turbidity upon each addition. Add copper chloride solution until the solution becomes slightly turbid, and add 0.1 mL in excess. Insert a stopper and shake for 1 minute. Add water to make exactly 10 mL and mix. Centrifuge this solution using a pointed centrifuge tube with a 15 mL graduation line. The presence of a 1 mm to 4 mm copper hydroxide precipitate is observed. Determine the absorption spectrum of the clear supernatant liquid as directed under Ultraviolet-visible Spectrophotometry in a 1 cm cell at 635 nm, using water as the blank, and subtract the absorbance of the blank solution (not more than 0.040) from the determined value to calculate the absorbances of the test solution and each of the standard solutions. Plot a calibration curve of the absorbance of each standard solution and from the calibration curve, determine the amount of glycerin in the test solution to calculate the amount of glycerin in the test specimen (not more than 5.5 %).

Sodium hydroxide solution—Dissolve 23.6 g of sodium hydroxide in water to make 100 mL.

Copper chloride solution—Dissolve 3.8 g of copper chloride in water to make 100 mL.

Water Not more than 5.0 % (0.1 g, coulometric titration). Use a water vaporizer (heating temperature: 105℃; heating time: 30 minutes).

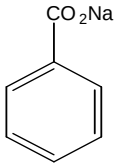
Assay Weigh accurately 25 mg of Sodium Aurothiomalate, previously dried, dissolve in 2 mL of aqua regia by heating and add water to make exactly 100 mL. Pipet 2 mL of the solution, add water to make exactly 25 mL and use this solution as the test solution. Pipet 5 mL, 10 mL and 15 mL of standard gold solution for atomic absorption spectrophotometry, add water to make exactly 25 mL and use these solutions as the standard solutions. Perform the test with the test solution and the standard solutions as directed under the Atomic Absorption Spectrophotometry under the following conditions. Determine the amount of gold in the test solution using the calibration curve obtained from the absorbances of the standard solutions.

Gas: Dissolved acetylene - Air
Lamp: A gold hollow cathode lamp.
Wavelength: 242.8 nm.

Containers and Storage ***Containers*****—**Tight containers.

Storage**—**Light-resistant.

Sodium Benzoate



$C_7H_5NaO_2$: 144.10

[*532-32-1*]

Sodium Benzoate, when dried, contains not less than 99.0 % and not more than 101.0 % of sodium benzoate ($C_7H_5NaO_2$).

Description Sodium Benzoate appears as white granules, crystals or crystalline powder, is odorless and has a sweet and saline taste.
Sodium Benzoate is freely soluble in water, slightly soluble in ethanol (95) and practically insoluble in ether.

Identification A solution of Sodium Benzoate (1 in 100) responds to the Qualitative Tests for benzoate and Qualitative Tests for (1) and (2) for sodium salt.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Sodium Benzoate in 5 mL of water: the solution is clear and colorless.

(2) ***Acid or alkali***—Dissolve 2.0 g of Sodium Benzoate in 20 mL of freshly boiled and cooled water and add 2 drops of phenolphthalein TS and 0.20 mL of 0.05 mol/L sulfuric acid VS: the solution remains colorless. To this solution, add 0.40 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(3) ***Sulfate***—Dissolve 0.40 g of Sodium Benzoate in 40 mL of water, add slowly 3.5 mL of dilute hydrochloric acid with thorough stirring, allow to stand for 5 minutes and filter. Discard the first 5 mL of the filtrate, take the subsequent 20 mL of the filtrate, add water to make 50 mL and perform the test. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.120 %).

(4) ***Heavy metals***—Dissolve 1.0 g of Sodium Benzoate in 44 mL of water, add gradually 6 mL of dilute hydrochloric acid with thorough stirring and filter. Discard the first 5 mL of the filtrate, take the subsequent 25 mL of the filtrate, neutralize with ammonia TS, add 2 mL of dilute acetic acid and water to make 50 mL and perform the test. Prepare the control solution as follows: to 1.0 mL of standard lead solution, add 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(5) ***Arsenic***—Mix well 1.0 g of Sodium Benzoate with 0.40 g of calcium hydroxide, ignite, dissolve the residue in 10 mL of dilute hydrochloric acid and perform the test (not more than 2 ppm).

(6) ***Chlorinated compounds***—Dissolve 1.0 g of Sodium Benzoate in 10 mL of water, add 10 mL of dilute sulfuric acid and extract with two 20 mL volumes of ether. Combine the ether extracts and evaporate the ether on a water-bath. Place 0.5 g of the residue and 0.7 g of calcium carbonate in a crucible, mix with a small amount of water and dry. Ignite it at about 600 °C, dissolve in 20 mL of dilute nitric acid and filter. Wash the residue with 15 mL of water, combine the filtrate and the washing, add water to make 50 mL and add 0.5 mL of silver nitrate TS: this solution has no more turbidity than the following control solution.

Control solution—Dissolve 0.7 g of calcium carbonate in 20 mL of dilute nitric acid and filter. Wash the residue with 15 mL of water, combine the filtrate and the washings, add 1.2 mL of 0.01 mol/L Hydrochloric acid VS and water to make 50 mL and add 0.5 mL of silver nitrate TS.

(7) ***Phthalic acid***—Dissolve exactly about 100 mg of Sodium Benzoate in 1 mL of water, add 1 mL of resorcinol-sulfuric acid TS, and heat in an oil bath at a temperature between 120 and 125 °C. After evaporating the water, heat again for 90 minutes, cool, and dissolve in 5 mL of water. To 1 mL of this solution add 10 mL of a solution of sodium hydroxide (43 in 500), and use this solution as the test solution. Separately, dissolve exactly 61 mg of potassium biphthalate in 1000 mL of water. Proceed with 1 mL of this solution in the same manner as the test solution, and use this solution as the standard solution. Determine the absorbances at 495 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry: the absorbance of the test solution is not more than that of the standard solution.

Loss on Drying Not more than 1.5 % (2 g, 110 °C, 4 hours).

Assay Weigh accurately 1.5 g of Sodium Benzoate, previously dried, and transfer to a glass-stoppered flask. Dissolve in 25 mL of water, add 75 mL of ether and 10 drops of bromophenol blue TS and titrate with 0.5 mol/L hydrochloric acid VS, while mixing the aqueous and ether layers by vigorous shaking, until a persistent, pale green color is produced in the aqueous layer.

Each mL of 0.5 mol/L hydrochloric acid VS
= 72.05 mg of $C_7H_5NaO_2$

Containers and Storage ***Containers***—Well-closed containers.

Sodium Bicarbonate

$NaHCO_3$: 84.01

Sodium hydrogen carbonate [*144-55-8*]

Sodium Bicarbonate contains not less than 99.0 % and not more than 101.0 % of sodium bicarbonate ($NaHCO_3$).

Description Sodium Bicarbonate appears as white crystals or crystalline powder, is odorless and has a characteristic, saline taste.
Sodium Bicarbonate is soluble in water and practically insoluble in ethanol (95) or in ether.
Sodium Bicarbonate slowly decomposes in moist air.

Identification A solution of Sodium Bicarbonate (1 in 30) responds to the Qualitative Tests for sodium salt and for bicarbonate.

pH Dissolve 1.0 g of Sodium Bicarbonate in 20 mL of water: the pH of this solution is between 7.9 and 8.4.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Sodium Bicarbonate in 20 mL of water: the solution is clear and colorless.

(2) ***Chloride***—To 0.40 g of Sodium Bicarbonate, add 4 mL of dilute nitric acid, heat to boil, cool and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test. Prepare the control solution with 0.45 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.040 %).

(3) ***Sulfide***—Weigh accurately 2.0 g of Sodium Bicarbonate, dissolve in 20 mL of water, boil to make 5 mL, add 1 mL of bromine TS, evaporate to dryness, and cool. Dissolve the residue in 10 mL of 3 mol/L hydrochloric acid TS, evaporate to dryness, and cool. Dissolve this residue in 5 mL of 3 mol/L hydrochloric acid TS, evaporate to dryness, and cool. To this residue add 10 mL of water, and adjust the pH to 2 with 3 mol/L hydrochloric acid TS or ammonia TS. If necessary to obtain a clear solution, filter this solution, wash the filter with two 2 mL volumes of water, combine, add water to make 20 mL, and use this solution as the test solution. Separately, to 0.30 mL of 0.01 mol/L sulfuric acid TS add 1 mL of 0.06 mol/l hydrochloric acid TS and water to make 20 mL, and use this solution as the standard solution. Add 1 mL each of barium chloride TS to the test solution and standard solution, mix, and allow to stand for 30 minutes: the turbidity of the test solution is not more intense than that of the standard solution (not more than 0.015 %).

(4) ***Carbonate***—Dissolve 1.0 g of Sodium Bicarbonate in 20 mL of freshly boiled and cooled water with very gentle swirling at a temperature not exceeding 15 °C. Add 2.0 mL of 0.1 mol/L hydrochloric acid VS and 2 drops of phenolphthalein TS: no red color develops immediately.

(5) ***Ammonium***—Heat 1.0 g of Sodium Bicarbonate: the gas evolved does not change moistened red litmus paper to blue.

(6) ***Heavy metals***—Dissolve 4.0 g of Sodium Bicarbonate in 5 mL of water and 4.5 mL of hydrochloric acid and evaporate on a water-bath to dryness. Dissolve the residue in 2 mL of dilute acetic acid, 35 mL of water and 1 drop of ammonium TS, dilute with water to make 50 mL and Perform the test. Prepare the control solution as follows: evaporate 4.5 mL of hydrochloric acid to dryness and add 2 mL of dilute acetic acid, 2.0 mL of standard lead solution and water to make 50 mL (not more than 5 ppm).

(7) ***Copper***—Transfer 5.0 g of Sodium Bicarbonate to a 100 mL plastic volumetric flask, add 4 mL of nitric acid, shake, sonicate for 30 minutes, add water to make 100 mL, and use this solution as the test solution. Separately, dissolve 1.0 g of Copper RS in 20 mL of nitric acid, and add 0.2 mol/L nitric acid to make 1000 mL. To 10 mL of this solution add 0.2 mol/L nitric acid to make 1000 mL, and use this solution as the standard stock solution. Keep the standard stock solution in a polyethylene bottle. To a suitable volume of the standard stock solution add 0.2 mol/L nitric acid so that each mL contains 0.1 μg, 0.05 μg, and 0.01 μg, and use these solutions as the standard solutions (1), (2), and (3), respectively. Perform the test with the test solution and standard solutions (1), (2), and (3) as directed under Atomic Absorption Spectrophotometry according to the following conditions, and determine the content of copper in the test solution using the calibration curve obtained from the standard solutions (not more than 1 ppm). Perform a blank determination using a solution of nitric acid (40 in 1000) and make any necessary correction.

Gas: Dissolved acetylene – Air
Lamp: Copper hollow cathode lamp
Wavelength: 324.7 nm

(8) ***Iron***—Perform the test when Sodium Bicarbonate is used in a preparation for hemodialysis. Weigh accurately 2.0 g of Sodium Bicarbonate, and neutralize with hydrochloric acid, noting the volume consumed. Transfer this solution to a 25 mL volumetric flask using a small amount of water, and use this solution as the test solution. Separately, transfer 1.0 mL of standard iron solution to a 25 mL volumetric flask, add the same volume of hydrochloric acid as used to prepare the test solution, and use this solution as the standard solution. Separately, transfer the same volume of hydrochloric acid as used to prepare the test solution into a 25 mL volumetric flask, and use as the blank solution. To each of the test solution, standard solution, and blank solution add 50 mg of ammonium peroxydisulfate, 2 mL of 30 % ammonium thiocyanate solution and water to make 25 mL. Determine the absorbances at 480 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry, using the blank solution as the blank: the absorbance of the test solution is not more than that of

the standard solution (not more than 5 ppm).

(9) ***Calcium***—Perform the test when Sodium Bicarbonate is used in a preparation for hemodialysis. Weigh accurately 3.0 g of Sodium Bicarbonate, add 6 mL of 6 mol/L hydrochloric acid TS and 1 g of potassium chloride, add water to make 100 mL, mix, and use this solution as the test solution. Separately, dissolve 0.25 g of calcium carbonate, previously dried at 300 °C for 3 hours and cooled in a desiccator for 2 hours, in 6 mL of 6 mol/L hydrochloric acid TS, add 1 g of potassium chloride, add water to make 100 mL, and mix. Pipet 10.0 mL of this solution, and add potassium chloride solution to make 100 mL so that each mL contains 100 μg of calcium. To 2.0 mL, 3.0 mL, 4.0 mL, and 5.0 mL of this solution add 6 mL of 6 mol/L hydrochloric acid TS, add potassium chloride solution to make 100 mL, and use these solutions as the 2.0, 3.0, 4.0, and 5.0 μg/mL calcium standard solutions. Perform the test with the test solution and standard solutions as directed under Atomic Absorption Spectrophotometry according to the following conditions, and determine the content of calcium in the test solution using the calibration curve obtained from the absorbances of the standard solutions: not more than 0.01 %.

Potassium chloride solution—Dissolve 10 g of potassium chloride in 1000 mL of 0.36 mol/L hydrochloric acid.

Gas: Dissolved acetylene – Nitrous oxide
Lamp: Calcium hollow cathode lamp
Blank solution: Potassium chloride TS
Wavelength: 422.7 nm

(10) ***Arsenic***—Dissolve 1.0 g of Sodium Bicarbonate in 3 mL of water and 2 mL of hydrochloric acid and perform the test (not more than 2 ppm).

Assay Weigh accurately about 2 g of Sodium Bicarbonate, dissolve in 100 mL of water and titrate with 1 mol/L hydrochloric acid VS. Slowly add 1 mol/L hydrochloric acid VS until a pale red-purple color develops, boil, cool, and continue the titration until the red-purple color does not disappear (indicator: 2 drops of methyl red TS).

Each mL of 1 mol/L sulfuric acid VS
= 84.01 mg of $NaHCO_3$

Containers and Storage ***Containers***—Tight containers.

Sodium Bicarbonate Injection

Sodium Bicarbonate Injection is an aqueous solution for injection. Sodium Bicarbonate Injection contains not less than 95.0 % and not more than 105.0 % of the labeled amount of sodium bicarbonate ($NaHCO_3$: 84.01).

Method of Preparation Prepare as directed under Injections, with Sodium Bicarbonate.

Description Sodium Bicarbonate Injection is a clear, colorless liquid.

Identification To a volume of Sodium Bicarbonate Injection, equivalent to 1 g of Sodium Bicarbonate according to the labeled amount, add water to make 30 mL: the solution responds to the Qualitative Tests for sodium salt and for bicarbonate.

Purify ***Carbonate—***To a volume of Sodium Bicarbonate Injection, equivalent to 0.10 g of Sodium Bicarbonate according to the labeled amount, add water, freshly boiled and cooled to 10 °C, to make a 1.0 w/v % solution and determine the pH immediately: the pH of this solution is between 7.9 and 8.6

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 5.0 EU/mEq of Sodium Bicarbonate Injection.

Insoluble Particulte Matter Test It meets the requirement.

Insoluble Particulte Matter Test for Injections It meets the requirement

Determination of Volume of Injection in Containers It meets the requirement.

Assay Measure exactly a volume of Sodium Bicarbonate Injection, equivalent to about 2 g of sodium bicarbonate ($NaHCO_3$), titrate with 1 mol/L hydrochloric acid VS and proceed as directed in the Assay under Sodium Bicarbonate.

Each mL of 1 mol/L hydrochloric acid VS
= 84.01 mg of $NaHCO_3$

Containers and Storage ***Containers***—Hermetic containers. Plastic containers for aqueous injections may be used.

Sodium Bicarbonate Tablets

Sodium Bicarbonate Tablets contain not less than 95.0 % and not more than 105.0 % of the labeled amount of sodium bicarbonate ($NaHCO_3$: 84.01)

Method of Preparation Prepare as directed under Tablets, with Sodium Bicarbonate.

Identification A portion of the powered tablets responds to the Identification under Sodium Bicarbonate.

Disintegration Test It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Sodium Bicarbonate Tablets. Weigh accurately a portion of powder, equivalent to about 2 g of sodium bicarbonate ($NaHCO_3$), dissolve in 100 mL of water and titrate with 1 mol/L hydrochloric acid VS. Slowly add 1 mol/L hydrochloric acid VS until a pale red-purple color develops, boil, cool, and continue the titration until the red-purple color does not disappear (indicator: 2 drops of methy red TS).

Each mL of 1 mol/L hydrochloric acid VS
= 84.01 mg of $NaHCO_3$

Containers and Storage ***Containers***—Well-closed containers.

Sodium Borate

$Na_2B_4O_7 \cdot 10H_2O$: 381.37

Disodium [oxido(oxoboranyloxy)boranyl]oxy-oxoboranyloxyborinate decahydrate [*1303-96-4*]

Sodium Borate contains not less than 99.0 % and not more than 103.0 % of sodium borate ($Na_2B_4O_7 \cdot 10H_2O$).

Description Sodium Borate appears as colorless or white crystals or a white, crystalline powder, is odorless and has a slightly characteristic, saline taste.
Sodium Borate is freely soluble in glycerin, soluble in water and practically insoluble in ethanol (95), in dehydrated ethanol (99.5) or in ether.
When placed in dry air, Sodium Borate effloresces and is coated with a white powder.

Identification A solution of Sodium Borate (1 in 20) responds to the Qualitative Tests for sodium salt and for borate.

pH Dissolve 1.0 g of Sodium Borate in 20 mL of water: the pH of this solution is between 9.1 and 9.6.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Sodium Borate in 20 mL of water by warming slightly: the solution is clear and colorless.

(2) ***Carbonate or bicarbonate***—Dissolve 1.0 g of powdered Sodium Borate in 20 mL of freshly boiled and cooled water and add 3 mL of dilute hydrochloric acid: the solution does not effervesce.

(3) ***Heavy metals***—Dissolve 1.5 g of Sodium Borate in 25 mL of water and 7 mL of 1 mol/L hydrochloric acid TS, add 1 drop of phenolphthalein TS and add ammonia TS until a pale red color develops. Then add dilute acetic acid until the solution becomes colorless again, add 2 mL of dilute acetic acid and add water to make 50 mL. Perform the test. Prepare the control solution as follows: to 3.0 mL of standard lead solution, add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(4) ***Arsenic***— Prepare the test solution with 0.40 g of Sodium Borate according to Method 1 and perform the test. (not more than 5 ppm).

Assay Weigh accurately about 2 g of Sodium Borate, dissolve in 50 mL of water and titrate with 0.5 mol/L hydrochloric acid VS (indicator: 3 drops of methyl red TS).

Each mL of 0.5 mol/L hydrochloric acid VS
= 95.34 mg of $Na_2B_4O_7.10H_2O$

Containers and Storage ***Containers***—Tight containers.

Sodium Bromide

NaBr: 102.89

[*7647-15-6*]

Sodium Bromide, when dried, contains not less than 99.0 % and not more than 101.0 % of sodium bromide (NaBr).

Description Sodium Bromide appears as colorless or white crystals or crystalline powder and is odorless.
Sodium Bromide is freely soluble in water and soluble in ethanol (95).
Sodium bromide is hygroscopic, but not deliquescent.

Identification A solution of Sodium Bromide (1 in 10) responds to the Qualitative Tests for sodium salt and for bromide.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Sodium Bromide in 3 mL of water: the solution is clear and colorless.

(2) ***Alkali***—Dissolve 1.0 g of Sodium Bromide in 10 mL of water, add 0.10 mL of 0.005 mol/L sulfuric acid VS and 1 drop of phenolphthalein TS, heat to boil and cool: the solution is colorless.

(3) ***Chloride***—Make a calculation from the result obtained in the Assay. Not more than 97.9 mL of 0.1 mol/L silver nitrate VS is consumed for 1 g of Sodium Bromide.

(4) ***Sulfate***—Perform the test with 2.0 g of Sodium Bromide. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024 %).

(5) ***Iodide***—Dissolve 0.5 g of Sodium Bromide in 10 mL of water, add 2 to 3 drops of iron (III) chloride TS and 1 mL of chloroform and shake: no red-purple to purple color develops in the chloroform layer.

(6) ***Bromate***—Dissolve 1.0 g of Sodium Bromide in 10 mL of freshly boiled and cooled water and add 2 drops of potassium iodide TS, 1 mL of starch TS and 3 drops of dilute sulfuric acid. Shake the mixture gently and allow to stand for 5 minutes: no blue color develops.

(7) ***Heavy metals***—Proceed with 2.0 g of Sodium Bromide according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(8) ***Magnesium and alkaline-earth metals***—To 200 mL of water add 0.1 g of hydroxylammonium hydrochloride, 10 mL of pH 10 ammonium chloride buffer solution, 1 mL of 0.1 mol/L zinc sulfate VS, and 0.2 g of eriochrome black T-sodium chloride indicator, and warm at 40 °C. To this solution add dropwise 0.01 mol/L disodium ethylenediaminetetraacetate VS until the red-purple color of the solution changes to blue-purple. To this solution add 10.0 g of Sodium Bromide dissolved in 100 mL of water. If the color changes to redd-purple, titrate the solution with 0.01 mol/L disodium ethylenediaminetetraacetate VS until the color of the solution changes to blue-purple: not more than 5.0 mL of 0.01 mol/L disodium ethylenediaminetetraacetate VS is consumed (not more than 0.02 % as calcium).

(9) ***Barium***—Dissolve 0.5 g of Sodium Bromide in 10 mL of water, add 0.5 mL of dilute hydrochloric acid and 1 mL of potassium sulfate TS and allow to stand for 10 minutes: no turbidity is produced.

(10) ***Iron***—Dissolve 0.5 g of Sodium Bromide in water to make 10 mL and use this solution as the test solution. To 1 mL of standard iron solution add water to make 10 mL, and use this solution as the standard solution. To the test solution and standard solution add 2.0 mL of citric acid solution (1 in 5) and 0.1 mL of thioglycolic acid, alkalify to litmus with ammonia solution (28), and add water to make 20 mL. After 5 minutes, the color of the test solution is not more intense than that of the standard solution (not more than 20 ppm).

(11) ***Arsenic***— Prepare the test solution with 1.0 g of Sodium Bromide according to Method 1 and perform the test (not more than 2 ppm).

Loss on Drying Not more than 5.0 % (1 g, 110 °C, 4 hours).

Assay Weigh accurately 0.4 g of Sodium Bromide, previously dried and dissolve in 50 mL of water. Add 10 mL of dilute nitric acid and 50 mL of 0.1 mol/L silver nitrate VS, exactly measured and titrate the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/ L silver nitrate VS
= 10.289 mg of NaBr

Containers and Storage ***Containers***—Tight containers.

Sodium Chloride

NaCl: 58.44

Sodium Chloride [*7647-14-5*]

Sodium Chloride, when dried, contains not less than 99.5 % and not more than 101.0 % of sodium chloride (NaCl).

Description Sodium Chloride appears as colorless or white crystals or crystalline powder.
Sodium Chloride is freely soluble in water, very slightly soluble in ethanol (99.5).

Identification (1) A solution of Sodium Chloride in water (1 in 20) responds to the Qualitative Tests for sodium salt.

(2) A solution of Sodium Chloride in water (1 in 20) responds to the Qualitative Tests for chloride.

Purity (1) ***Clarity and color of solution***— Dissolve 1.0 g of Sodium Chloride in 5 mL of water: the solution is clear and colorless.

(2) ***Acidity or alkalinity***— Dissolve 20.0 g of Sodium Chloride in 100.0 mL of freshly boiled and cooled water, and use this solution as the test solution. To 20 mL of the test solution, add 0.1 mL of bromthymol blue TS and 0.5 mL of 0.01 mol/L hydrochloric acid VS: the color of the solution is yellow. Separately, to 20 mL of the test solution add 0.1 mL of bromothymol blue TS and 0.5 mL of 0.01 mol/L sodium hydroxide VS: the color of the solution is blue.

(3) ***Bromides***—To 0.50 mL of the test solution obtained in (2) add 4.0 mL of water, 2.0 mL of dilute phenolred TS and 1.0 mL of a solution of sodium toluenesulfonchloramide trihydrate (1 in 10000), and mix immediately. After 2 minutes, add 0.15 mL of 0.1 mol/L sodium thiosulfate VS, mix, add water to make exactly 10 mL, and use this solution as the test solution. Separately, to 5.0 mL of a solution of potassium bromide (3 in 1000000) add 2.0 mL of dilute phenol red TS and 1.0 mL of a solution of sodium toluenesulfonchloramide trihydrate (1 in 10000), and mix immediately. Proceed in the same manner as for the preparation of the test solution, and use the solution so obtained as the standard solution. Perform the test with the test solution and the standard solution, respectively, as directed under Ultraviolet-visible Spectrohotometry using water as the control: the absorbance at 590 nm of the test solution is not more than that of the standard solution.

(4) ***Nitrite***—Weigh accurately 20.0 g of Sodium Chloride, and dissolve in water containing no carbon dioxide to make 100.0 mL. To 10 mL of this solution add 10 mL of water, and determine the absorbance at

354 nm of this solution as directed under Ultraviolet-visible Spectrophotometry: not more than 0.01.

(5) ***Iodides***—Wet 5 g of Sodium Chloride with dropwise addition of 0.15 mL of a freshly prepared mixture of starch TS, 0.5 mol/L sulfuric acid TS and sodium nitrite TS (1000 : 40 : 3), allow to stand for 5 minutes, and examine under daylight: a blue color does not appear.

(6) ***Phosphates***—To 2.0 mL of the test solution obtained in (2) add 5 mL of 2 mol/L sulfuric acid TS and water to make 100.0 mL, then add 4 mL of ammonium molybdate-sulfuric acid TS and 0.1 mL of tin (II) chloride-hydrochloric acid TS, and allow to stand for 10 minutes: the color of the solution is not darker than the following control solution.

Control solution—To 1.0 mL of Standard Phosphoric acid Solution add 12.5 mL of 2 mol/L sulfuric acid TS and water to make exactly 250 mL. To 100 mL of this solution add 4 mL of ammonium molybdate-sulfuric acid TS and 0.1 mL of tin (II) chloride-hydrochloric acid TS, and allow to stand for 10 minutes.

(7) ***Sulfates***—To 7.5 mL of the test solution obtained in (2) add water to make 30 mL, and use this solution as the test solution. Separately, dissolve 0.181 g of the potassium sulfate in diluted dehydrated ethanol (3 in 10) to make exactly 500 mL. Pipet 5 mL of this solution, and add diluted dehydrated ethanol (3 in 10) to make exactly 100 mL. To 4.5 mL of this solution add 3 mL of a solution of barium chloride dihydrate (1 in 4), shake, and allow to stand for 1 minute. To 2.5 mL of this solution add 15 mL of the test solution and 0.5 mL of acetic acid, and allow to stand for 5 minutes: any turbidity produced is not more than that produced in the following control solution.

Control solution—Dissolve 0.181 g of potassium sulfate in water to make exactly 500 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and proceed in the same manner as directed above using this solution instead of the test solution

(8) ***Ferrocyanides***—Dissolve 2.0 g of Sodium Chloride in 6 mL of water, and add 0.5 mL of a mixture of a solution of iron (II) sulfate heptahydrate (1 in 100) and a solution of ammonium iron (III) sulfate dodecahydrate in diluted sulfuric acid (1 in 400) (1 in 100) (19 : 1): a blue color does not develop within 10 minutes.

(9) ***Heavy metals***—Proceed with 5.0 g of Sodium Chloride according to Method 1 and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 3 ppm).

(10) ***Magnesium and alkaline-earth materials***—To 200 mL of water add 0.1 g of hydroxylammonium chloride, 10 mL of ammonium chloride buffer solution, pH 10, 1 mL of 0.1 mol/L zinc sulfate VS and 0.2 g of eriochrome black T-sodium chloride indicator, and warm to 40 °C. Add 0.01 mol/L disodium dihdrogen ethylenediamine tetraacetate VS dropwise until the red-purple color of the solution changes to blue-purple. To this solution add a solution prepared by dissolving 10.0 g of Sodium Chloride in 100 mL of water, and add 2.5 mL of 0.01 mol/L disodium ethylenediamine tetraacetate VS: the color of the solution is a blue-purple.

(11) ***Barium***—To 5.0 mL of the test solution obtained in (2) add 5.0 mL of water and 2.0 mL of dilute sulfuric acid, and allow to stand for 2 hours: the solution has not more turbidity than the following control solution.

Control solution—To 5.0 mL of the test solution obtained in (2) add 7.0 mL of water, and allow to stand for 2 hours.

(12) ***Aluminum***—Perform this test when Sodium Chloride is used in preparations for peritoneal dialysis, hemodialysis, or hemofiltration. Weigh accurately 20.0 of Sodium Chloride, dissolve in 100 mL of water, add 10 mL of acetic acid-ammonium acetate buffer solution (pH 6.0), extract with successive portions of 20 mL, 20 mL, and 10 mL of a 0.5 % solution of 8-hydroxyquinoline in chloroform, combine the extracts in a 50 mL volumetric flask, add chloroform to make 50 mL, and use this solution as the test solution. Separately, dissolve 0.352 g of aluminum potassium sulfate in a small amount of water, add 20 mL of dilute sulfuric acid, and add water to make 100 mL. Immediately before use, pipet 1.0 mL of this solution, and add wter to make 100 mL. To 2.0 mL of this solution add 10 mL of acetic acid-ammonium acetate buffer solution (pH 6.0) and 98 mL of water, extract in the same manner as the test solution, combine the extracts in a 50 mL volumetric flask, add chloroform to make 50 mL, and use this solution as the standard solution. To 10 mL of acetic acid-ammonium acetate buffer solution (pH 6.0) add 100 mL of water, extract with chloroform in the same manner as the test solution, and use as the blank solution. Perform the test with the test solution and standard solution as directed under Fluorimetry, and determine the fluorescence at an excitation wavelength of 392 nm and a detection wavelength of 518 nm: the fluorescence of the test solution is not larger than that of the standard solution (0.2 μg/g).

(13) ***Iron***—To 10 mL of the test solution from (2) add 2 mL of a solution of citric acid monohydrate (1 in 5) and 0.1 mL of mercaptoacetic acid, alkalify with ammonia TS, and add water to make 20 mL. Allow to stand for 5 minutes: the color of the solution is not more intense than the following control solution.

Control solution—Pipet 1 mL of standard iron solution, and add water to make exactly 25 mL. To 10 mL of this solution add 2 mL of a solution of citric acid monohydrate (1 in 5) and 0.1 mL of mercaptoacetic acid, and proceed in the same manner.

(14) ***Potassium***—Perform this test when Sodium Chloride is used in preparations for injection, peritoneal dialysis, hemodialysis, or hemofiltration. Weigh accurately 1.00 g of Sodium Chloride, add water to make 100 mL, mix well to sissolve, and use this solution as the test solution. Separately, weigh accurately 1.144 g of potassium chloride, previously dried at 105 °C for 3 hours, dissolve in water to make 1000 mL, make a solution so that each mL contains 600 μg of potassium, and use this solution as the standard stock solution. Dilute if necessary, and use as the standard stock solution. Perform the test with the test solution and standard solution as directed under Atomic Absorption Spectrophotometry not less than 3 times according to the following conditions, and calculate the content of potassium in the test solution using the calibration curve obtained from the absorbances of the standard solution: not more than 0.05 %.

Gas: Dissolved acetylene – Air
Wavelength: 766.5 nm

(15) ***Arsenic***—Prepare the test solution with 2.0 g of Sodium Chloride according to Method 1 and perform the test (not more than 1 ppm).

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 2 hours).

Assay Weigh accurately about 50 mg of Sodium Chloride, previously dried, dissolve in 50 mL of water and titrate with 0.1 mol/L silver nitrate VS (potentiometric titration, Endpoint Detection Method in Titrimetry).

Each mL of 0.1 mol/L silver nitrate VS
= 5.844 mg of NaCl

Containers and Storage ***Containers***—Tight containers.

10 % Sodium Chloride Injection

10 % Sodium Chloride Injection is an aqueous solution for injection.
10 % Sodium Chloride Injection contains not less than 9.5 w/v % and not more than 10.5 w/v % of sodium chloride (NaCl: 58.44).

Method of preparation

Sodium Chloride	100 g
Water for Injection	a sufficient quantity
	To make 1000 mL

Prepare as directed under Injections, with the above ingredients.

Description 10 % Sodium Chloride Injection is a clear, colorless liquid and has a saline taste.
10 % Sodium Chloride Injection is neutral.

Identification 10 % Sodium Chloride Injection responds to the Qualitative Tests for sodium salt and for chloride.

Bacterial Endotoxins Less than 3.6 EU/mL.

Sterility Test It meets the requirement.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay Pipet 10 mL of 10 % Sodium Chloride Injection and add water to make exactly 100 mL. Pipet 20 mL of this solution, add 30 mL of water and titrate with 0.1 mol/L silver nitrate VS while shaking vigorously (indicator: 3 drops of fluorescein sodium TS).

Each mL of 0.1 mol/L silver nitrate VS
= 5.844 mg of NaCl

Containers and Storage ***Containers***—Hermetic containers. Plastic containers for aqueous injections may be used.

Isotonic Sodium Chloride Injection

0.9 % Sodium Chloride Injection
Isotonic Salt Solution

Isotonic Sodium Chloride Injection is an aqueous solution for injection. Isotonic Sodium Chloride Injection contains not less than 0.85 % and not more than 0.95 % of sodium chloride (NaCl: 58.44).

Method of prepration

Sodium Chloride	9 g
Water for Injection	a sufficient quantity
	To make 1000 mL

Prepare as directed under Injections, with the above ingredients.
No preservative is added.

Description Isotonic Sodium Chloride Injection is a clear, colorless liquid and has a slightly saline taste.

Identification Isotonic Sodium Chloride Injection responds to the Qualitative Tests for sodium salt and for chloride.

pH 4.5 ~ 8.0.

Purity (1) ***Heavy metals***—Concentrate 100 mL of Isotonic Sodium Chloride Injection to about 40 mL in a water-bath, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 3.0 mL of standard lead solution and 2 mL of dilute acetic acid, and add water to make 50 mL (not more than 0.3 ppm).

(2) ***Arsenic***—Prepare the test solution with 20 mL of Isotonic Sodium Chloride Injection, and perform the test (not more than 0.1 ppm).

Sterility Test It meets the requirement.

Bacterial Endotoxins Not more than 0.50 EU/mL of Isotonic Sodium Chloride Injection.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Iinjection It meets the requirement.

Determination of Volume of Injection in Container It meets the requirement.

Assay Measure exactly 20 mL of Isotonic Sodium Chloride Injection, add 30 mL of water, and titrate with 0.1 mol/L silver nitrate VS with vigorous shaking (indicator: 3 drops of fluorescein sodium TS).

Each mL of 0.1 mol/L silver nitrate VS
= 5.844 mg of NaCl

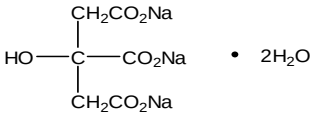
Containers and Storage ***Containers***—Hermetic containers. Plastic containers for aqueous injections may be used.

Sodium Chromate (^{51}Cr) Injection

Sodium Chromate (^{51}Cr) Injection is an aqueous solution for injection.
Sodium Chromate (^{51}Cr) Injection contained ^{51}Cr in the form of Sodium Chromate.
Sodium Chromate (^{51}Cr) Injection meets the requirement of Sodium Chromate (^{51}Cr) Injection of the Korean Pharmaceutical Codex.
The Test for Exractable Volume of Parenteral Preparations and the Insoluble Particulate Matter Test for Injections is not applied to this injection.

Description Sodium Chromate (^{51}Cr) Injection is a clear, colorless to pale yellow liquid and is odorless or has an odor of the preservatives.

Sodium Citrate Hydrate



$C_6H_5Na_3O_7 \cdot 2H_2O$: 294.10

Trisodium 2-hydroxypropane-1,2,3-tricarboxylate dihydrate [*6132-04-3*]

Sodium Citrate Hydrate, when dried, contains not less than 99.0 % and not more than 101.0 % of sodium citrate ($C_6H_5Na_3O_7$: 258.07).

Description Sodium Citrate Hydrate appears as colorless crystals or a white crystalline powder, is odorless and has a cooling saline taste.
Sodium Citrate Hydrate is freely soluble in water and practically insoluble in ethanol or in ether.

Identification A solution of Sodium Citrate Hydrate in water (1 in 20) responds to the Qualitative Tests for citrate and for sodium salt.

pH Dissolve 1.0 g of Sodium Citrate Hydrate in 20 mL of water: the pH of this solution is between 7.5 and 8.5.

Purity (1) ***Clarity and color of solution***—A solution of 1.0 g of Sodium Citrate Hydrate in 10 mL of water is clear and colorless.

(2) ***Chloride***—Weigh 0.6 g of Sodium Citrate Hydrate and perform the test. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.015 %).

(3) ***Sulfate***—To 0.5 g of Sodium Citrate Hydrate, add water to make 40 mL, then add 3.0 mL of dilute hydrochloric acid and water to make 50 mL and perform the test. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048 %).

(4) ***Heavy metals***—Proceed with 2.5 g of Sodium Citrate Hydrate according to Method 2 and perform the test. Prepare the control solution with 2.5 mL of standard lead solution (not more than 10 ppm).

(5) ***Arsenic***— Prepare the test solution with 1.0 g of Sodium Citrate Hydrate according to Method 1 and perform the test (not more than 2 ppm).

(6) ***Tartrate***—To 1.0 g of Sodium Citrate Hydrate, add 2 mL of water, 1 mL of potassium acetate TS and 1 mL of acetic acid (31): no crystalline precipitate is formed after the sides of the tube have been rubbed with a glass rod.

(7) ***Oxalate***—Dissolve 1.0 g of Sodium Citrate Hydrate in a mixture of 1 mL of water and 3 mL of dilute hydrochloric acid, add 4 mL of ethanol (95) and 0.2 mL of calcium chloride TS and allow to stand for 1 hour: the solution is clear.

(8) ***Readily carbonizable substances***—Weigh 0.5 g of Sodium Citrate Hydrate and perform the test by heating at 90 °C for 1 hour: the solution has no more color than Color Matching Fluid K.

Loss on Drying 10.0 ~ 13.0 % (1 g, 180 °C, 2 hours).

Assay Weigh accurately about 0.1 g of Sodium Citrate Hydrate, previously dried, dissolve in water, add exactly 10 mL of internal standard solution, add water to make exactly 100 mL and use this solution as the test solution. Separately, weigh accurately 0.1 g of Sodium Citrate Hydrate RS, previously dried, dissolve in water, add exactly 10 mL of internal standard solution and water to make exactly 100 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratio, Q_T and Q_S, of the peak area of Sodium Citrate to that of the internal standard for the test solution and the standard solution, respectively,

$$\text{Amount (mg) of Sodium Citrate } (C_6H_5Na_3O_7) = \text{Amount (mg) of sodium citrate in Sodium Citrate Hydrate RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—Acetic acid solution (1 in 100).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column**:** A stainless steel column, about 4 mm in internal diameter and about 15 cm to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography.

Mobile phase: Dissolve 2.64 g of diammonium hydrogen phosphate and 2 mL of triethylamine in 1000 mL of water and adjust the pH to 2.5 with phosphoric acid.

Flow rate: Adjust the flow rate to make the retention time of Sodium Citrate being about 5 minutes.

Selection of column: proceed with 20 μL of the standard solution under the above operating conditions. Use a column giving elution of the internal standard and sodium citrate in this order with the resolution between their peaks being not less than 2.0.

Containers and Storage ***Containers***—Tight containers.

Sodium Citrate Injection for Transfusion

Sodium Citrate Injection for Transfusion is an aqueous solution for injection. Sodium Citrate Injection for Transfusion contains not less than 9.5 w/v % and not more than 10.5 w/v % of sodium citrate hydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$: 294.10).

Method of preparation

Sodium Citrate Hydrate	100 g
Water for injection	a sufficient quantity
To make	1000 mL

Prepare as directed under Injections, with the above ingredients.
No preservative is added.

Description Sodium Citrate Injection for Transfusion is a clear, colorless liquid.

Identification Sodium Citrate Injection for Transfusion responds to the Qualitative Tests for sodium salt and for citrate.

pH 7.0 ~ 8.5.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 5.6 EU/mL of Sodium Citrate Injection for Transfusion.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

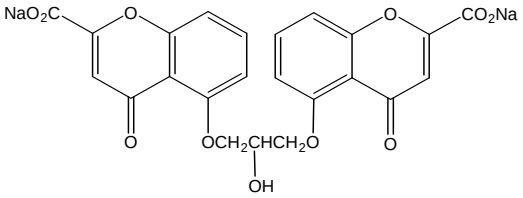
Determination of Volume of Injection in Containers It meets the requirement.

Assay Pipet 5.0 mL of Sodium Citrate Injection for Transfusion, add water to make exactly 25 mL, pipet 10.0 mL of this solution and evaporate on a water-bath to dryness. Dry the residue at 180 °C for 2 hours and dissolve in 30 mL of acetic acid (100) by warming. After cooling, titrate with 0.1 mol/L perchloric acid VS (indicator: 3 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

$$\text{Each mL of 0.1 mol/L perchloric acid VS} = 9.803 \text{ mg of } C_6H_5Na_3O_7.2H_2O$$

Containers and Storage ***Containers***—Hermetic containers.

Sodium Cromoglicate



$C_{23}H_{14}Na_2O_{11}$: 512.33

Disodium 5-[3-(2-carboxylato-4-oxochromen-5-yl)oxy-2-hydroxypropoxy]-4-oxochromene-2-carboxylate [*15826-37-6*]

Sodium Cromoglicate contains not less than 98.0 % and not more than 101.0 % of sodium cromoglicate ($C_{23}H_{14}Na_2O_{11}$), calculated on the dried basis.

Description Sodium Cromoglicate is a white, crystalline powder, is odorless and tasteless at first and later develops a slightly bitter taste.
Sodium Cromoglicate is freely soluble in water, sparingly soluble in propylene glycol, very slightly soluble in ethanol (95) and practically insoluble in 2-propanol or in ether.
Sodium Cromoglicate is hygroscopic.
Sodium Cromoglicate gradually acquire a yellow color by light.

Identification (1) Dissolve 0.1 g of Sodium Cromoglicate in 2 mL of water, add 2 mL of sodium hydroxide TS and boil for 1 minute: a yellow color is observed. After cooling, add 0.5 mL of concentrated diazobenzene sulfonic acid TS: a dark red color is observed.

(2) Determine the absorption spectra of solutions of Sodium Cromoglicate and Sodium Cromoglicate RS in phosphate buffer solution, pH 7.4, (1 in 100000), as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Sodium Cromoglicate responds to the Qualitative Tests for sodium salt.

Purity (1) ***Clarity and color of solution***—Dissolve 0.50 g of Sodium Cromoglicate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) ***Acid or alkali***—Dissolve 2.0 g of Sodium Cromoglicate in 40 mL of freshly boiled and cooled water, add 6 drops of bromothymol blue TS and use this solution as the test solution. To 20 mL of the test solution, add 0.25 mL of 0.1 mol/L sodium hydroxide VS: a blue color is observed. To another 20 mL of the test solution, add 0.25 mL of 0.1 mol/L hydrochloric acid VS: a yellow color is observed.

(3) ***Heavy metals***—Proceed with 1.0 g of Sodium Cromoglicate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Oxalate***—Dissolve 0.25 g of Sodium Cromoglicate in water to make exactly 50 mL and use this solution as the test solution. Separately, dissolve 49 mg of oxalic acid, exactly weighed, in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL and use this solution as the standard solution. Pipet 20 mL each of the test solution and the standard solution, add 5 mL of iron salicylate TS to each solution and add water to make exactly 50 mL. Determine the absorbance of these solutions as directed under Ultraviolet-visible Spectrophotometry using water as a blank: the absorbance of the test solution at 480 nm is not smaller than that of the standard solution.

(5) ***Related substances***—Dissolve 0.20 g of Sodium Cromoglicate in 10 mL of water and use this solution as the test solution. Pipet 1 mL of the test solution, add water to make exactly 10 mL, pipet 1 mL of this solution, add water to make exactly 20 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, Develop the plate with a mixture of methanol, chloroform and acetic acid (100) (9 : 9 : 2) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): spots other than the principal spot from the test solution is not more intense than the spot from the standard solution.

Loss on Drying Not more than 10.0 % (1 g, in vacuum, 105 °C, 4 hours).

Assay Weigh accurately about 0.18 g of Sodium Cromoglicate and dissolve in a mixture of 25 mL of propylene glycol and 5 mL of 2-propanol by warming. After cooling, add 30 mL of 1,4-dioxane and titrate with 0.1 mol/L perchloric acid-1,4-dioxane VS (potentiometric titration, Endpoint Detection method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid-1,4-dioxane VS
= 25.62 mg of $C_{23}H_{14}Na_2O_{11}$

Containers and Storage ***Containers***—Tight containers.
Storage—Light-resistant.

Sodium Fluoride

NaF: 41.99

[*7681-49-4*]

Sodium Fluoride contains not less than 98.0 % and not

more than 102.0 % of sodium fluoride (NaF), calculated on the dried basis.

Description Sodium Fluoride appears as white powder and is odorless.
Sodium Fluoride is soluble in water and practically insoluble in ethanol (95).

Identification (1) Place 1 g of Sodium Fluoride in a platinum crucible, add 15 mL of sulfuric acid and cover the crucible with a glass plate. Heat the crucible in a water-bath for 1 hour, remove the glass cover, rinse it in water and wipe dry: the surface of the glass is etched.

(2) A solution of Sodium Fluoride (1 in 25) responds to the Qualitative Tests for sodium salt.

Purity (1) ***Acid or Alkali***—Dissolve 2.0 g of Sodium Fluoride in 40 mL of water in a platinum dish, add 10 mL of a saturated solution of potassium nitrate, cool the solution to 0 °C and add 3 drops of phenolphthalein TS. If no color appears, a pale red color persisting for 15 seconds is produced by not more than 2.0 mL of 0.10 mol/L sodium hydroxide VS. If the solution is colored pale red by the addition of phenolphthalein TS, it is rendered colorless by not more than 0.50 mL of 0.05 mol/L sulfuric acid. Save the neutralized solution for the test for the Purity (2).

(2) ***Fluosilicate***—After the solution obtained from Purity (1) has been neutralized, heat to boiling and titrate while hot with 0.10 mol/L sodium hydroxide VS until a permanent pale red color is obtained: not more than 1.5 mL of 0.10 mol/L sodium hydroxide VS is required.

(3) ***Chloride***—Dissolve 0.3 g in 20 ml of water, add 0.2 g of boric acid, 1 mL of nitric acid and 1 mL of 0.1 mol/L silver nitrate VS: It has no more color than the following control solution (not more than 0.012 %).

Control solution—To 1 mL of 0.0010 mol/L hydrochloric acid VS, add 0.2 g of boric acid, 1 mL of nitric acid and 1 mL of 0.1 mol/L silver nitrate VS.

(4) ***Heavy metals***—To 1.0 g of Sodium Fluoride, in platinum crucible, add 1 mL of water and 3 mL of sulfuric acid and heat at as low a temperature as practicable until all of the sulfuric acid has been expelled. Dissolve the residue in 20 mL of water, neutralize the solution with ammonia solution (28) using phenolphthalein TS as an indicator, add 1 mL of acetic acid (100), dilute with water to make 45 mL, filter and perform the test with 30 mL of the filtrate as a test solution. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

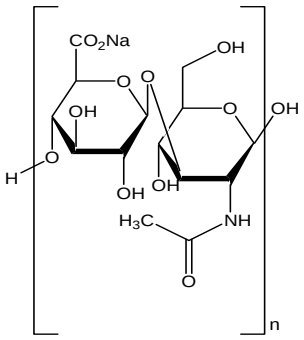
Loss on Drying Not more than 1.0 % (1 g, 150 °C, 4 hours).

Assay Weigh accurately about 80 mg of Sodium Fluoride, dissolve in 25 mL of mixture solution of acetic anhydride and acetic acid (100) (1 : 4), and titrate with 0.1 mol/L perchloric acid VS (indicator : methylrosaniline chloride TS) until the color of the solution changes into green. Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 4.199 mg of NaF

Containers and Storage ***Containers***—Well-closed containers.

Sodium Hyaluronate



$(C_{14}H_{20}NNaO_{11})_n$

Sodium (2*S*,3*S*,4*R*,5*R*,6*R*)-3-{[(2*S*,3*R*,5*S*,6*R*)-3-(acetylamino)-5-hydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl]oxy}-4,5,6-trihydroxytetrahydro-2*H*-pyran-2-carboxylate
[*9067-32-7*]

Sodium Hyaluronate, extracted from cocks' combs or obtained by fermentation from *Streptococci* Lancefield Groups A and C, is produced by methods of minimizing or eliminating infectious agents. When Sodium Hyaluronate is produced by fermentation of gram-positive bacteria, the process that reduces or eliminates pyrogenic or inflammatory components of the cell wall must be specified.
Sodium Hyaluronate is the sodium salt of hyaluronic acid, a glycosaminoglycan consisting of D-glucuronic acid and *N*-acetyl-D-glucosamine disaccharide units.
Sodium Hyaluronate contains not less than 95.0 % and not more than 105.0 % of sodium hyaluronate $(C_{14}H_{20}NNaO_{11})_n$, calculated on the dried basis. Sodium Hyaluronate has an intrinsic viscosity of not less than 90 % and not more than 120 % of the value stated on the label.

Description Sodium Hyaluronate appears as white powder, granules, or fibrous masses.
Sodium Hyaluronate is sparingly soluble in water and practically insoluble in ethanol (95).
Sodium Hyaluronate is hygroscopic.

Identification (1) Determine the infrared spectra of Sodium Hyaluronate and Sodium Hyaluronate RS as

directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) A solution of Sodium Hyaluronate (1 in 20) responds to the Qualitative Tests for sodium salt

pH Dissolve a portion of Sodium Hyaluronate, equivalent to 50 mg on the dried basis, in 10 mL of water: the pH of this solution is between 5.0 and 8.5.

Intrinsic Viscosity Sodium Hyaluronate is highly hygroscopic and must be protected from moisture during weighing. Weigh accurately 0.200 g (m_{0p}) of Sodium Hyaluronate (this weight is an only indicative value and should be adjusted after an initial measurement of the viscosity of the test solution (1)), add 50 g (m_{0s}) of buffer solution at 4 °C and mix by shaking at 4 °C for 24 hours. Weigh accurately 5.00 g (m_{1p}) of this solution, add 100.0 g of buffer solution (m_{1s}) at 25 °C, mix by shaking for 20 minutes and filter this solution with glass filter. Discard the first 10 mL of the filtrate and use the remaining filtrate as the test solution (1) (the concentration of Sodium Hyaluronate in the test solution (1) as C_1). Weigh accurately 30.0 g (m_{2p}) of the test solution (1), add 10.0 g (m_{2s}) of buffer solution at 25 °C, mix by shaking for 20 minutes and filter this solution with glass filter. Discard the first 10 mL and use the remaining filtrate as the test solution (2) (the concentration of Sodium Hyaluronate in the test solution (2) as C_2). Weigh 20.0 g (m_{3p}) of the test solution (1), add 20.0 g (m_{3s}) of buffer solution at 25 °C, mix by shaking for 20 minutes and filter this solution with glass filter. Discard the first 10 mL and the remaining filtrate as the test solution (3) (the concentration of Sodium Hyaluronate in the test solution (3) as C_3). Weigh 10.0 g (m_{4p}) of the test solution (1), add 30.0 g (m_{4s}) of buffer solution at 25 °C, mix by shaking for 20 minutes and filter this solution with glass filter. Discard the first 10 mL and the remaining filtrate as the test solution (4) (the concentration of Sodium Hyaluronate in the test solution (4) as C_4). Determine the downflowing time for the test solution (1), (2), (3), (4) and buffer solution, t_1, t_2, t_3, t_4 and t_0, respectively, at 25.00 ± 0.03 °C. Use the same viscometer (viscometer constant 0.005 mm^2/s^2, kinematic viscosity between 1 and 5 mm^2/s^2, internal diameter of tube below the bulb C 0.53 mm, volume of bulb B 5.6 mL, internal diameter of tube 2 between 2.8 mm and 3.2 mm) for all measurements. Measure the downflowing time in triplicate from the upper marked line to the lower marked line of the bulb B. The test is not valid if the results differ by more than 0.35 % from the mean and the downflowing time t_1 is not less than 1.6 and not more than 1.8 times of t_0. If the test is not valid, adjust the value of m_{0p} and retest.

Calculation of the relative viscosities—Since the densities of the sodium hyaluronate solutions and of the solvent are almost equal, calculate the relative viscosities η_{ri} (η_{r1}, η_{r2}, η_{r3} and η_{r4}) from the ratio of the downflowing times for the respective solutions t_i (t_1, t_2, t_3 and t_4) to the downflowing time of the solvent t_0 by the following equation.

$$\eta_{ri} = \frac{t_i - \dfrac{B}{t_i^2}}{t_0 - \dfrac{B}{t_0^2}}$$

B: Correction factor (30800 s^3) for kinetic energy for the capillary

Calculation of C_1—The concentration (kg/m^3) of Sodium Hyaluronate in the test solution (1) (C_1).

$$C_1 = m_{0p} \times \frac{x}{100} \times \frac{100 - h}{100} \times \frac{1}{m_{0p} + m_{0s}} \times \frac{m_{1p}}{m_{1p} + m_{1s}} \times \rho_{25}$$

x: Content (%) of sodium hyaluronate as determined in the Assay

h: Loss on Drying in %

ρ_{25}: 1005 kg/m^3 (the density of the test solution at 25 °C)

Calculation of the other concentrations

$$C_2 = C_1 \times \frac{m_{2p}}{m_{2p} + m_{2s}}$$

$$C_3 = C_1 \times \frac{m_{3p}}{m_{3p} + m_{3s}}$$

$$C_4 = C_1 \times \frac{m_{4p}}{m_{4p} + m_{4s}}$$

Calculation of the intrinsic viscosity—The intrinsic viscosity [η] (m^3/kg) is calculated by linear least-squares regression analysis using the Martin equation and by taking the decimal antilogarithm of the intercept.

$$\log\{(\eta_r - 1)/C\} = \log[\eta] + k \cdot [\eta] \cdot C$$

Buffer solution—Weigh 0.78 g of sodium dihydrogen phosphate and 4.50 g of sodium chloride, add water to make 500 mL and use this solution as the solution A. Weigh 1.79 g of disodium hydrogen phosphate and 4.50 g of sodium chloride, add water to make 500 mL and use this solution as the solution B. Mix solutions A and B, adjust pH to 7.0 and filter the solution with glass filter.

Purity (1) ***Clarity and color of solution***—Dissolve a portion of Sodium Hyaluronate, equivalent to 0.1 g of sodium hyaluronate on the dried basis, in 30 mL of 0.9 w/v % sodium chloride solution by a gentle shaking for 12 hours: the solution is clear. Determine the absorbance of this solution at 600 nm as directed under Ultraviolet-visible Spectrophotometry: it is not more than

0.01.

(2) ***Chloride***—Weigh 67 mg of Sodium Hyaluronate, and add 100 mL of water. Pipet 15 mL of this solution, add 1 mL of dilute nitric acid and 1 mL of silver nitrate TS. Perform the test using this solution as the test solution. Prepare the control solution by mixing 10 mL of standard chloride solution, 5 mL of water, 1 mL of dilute nitric acid and 1 mL of silver nitrate TS (not more than 0.5 %).

Standard chloride solution—Weigh accurately 0.824 g of sodium chloride, add water to make exactly 1000 mL. To 1 mL of this solution, add water to make exactly 100 mL immediately before use.

(3) ***Heavy metals***—Transfer 1.0 g of Sodium Hyaluronate to a 100 mL combustion flask, add a mixture of nitric acid and sulfuric acid (5 : 4) until the sample is thoroughly moistened, and heat gently. Repeat this procedure until 18 mL of the mixture of nitric acid and sulfurc acid (5 : 4) has been added. Boil gently until the color of the solution becomes black. After cooling, add 2 mL of nitric acid, and heat again until the color of the solution becomes black. Repeat this procedure until the color of the solution does not become black, then heat strongly until dense, white fumes are produced. After cooling, add 5 mL of water, boil gently until dense, white fumes are produced, and continue heating to reduce to 2 to 3 mL. After cooling, add 5 mL of water. If the color of the solution is yellow, add 1 mL of strong hydrogen peroxide, and heat to reduce to 2 to 3 mL. After cooling, dilute with 2 to 3 mL of water, transfer to a Nessler tube, add water to make 25 mL, and use this solution as the test solution. Separately, transfer 2.0 mL of standard lead solution to a 100 mL combustion flask, add 18 mL of a mixture of nitric acid and sulfuric acid (5 : 4) and the same amount of nitric acid used to prepare the test solution, and heat until dense, white fumes are produced. After cooling, add 10 mL of water. If strong hydrogen peroxide was used to prepare the test solution, add the same amount. Then, proceed in the same manner as the test solution, and use this solution as the control solution. Adjust the pH of the test solution and control solution to 3.0 to 4.0 with ammonia solution (28), and add water to make 40 mL. To each solution add 1.2 mL of thioacetamide TS, 2 mL of pH 3.5 acetate buffer solution, and water to make 50 mL, allow to stand for 5 minutes, and examine the colors of the solutions against a white background: the color of the test solution is not more intense than that of the control solution (not more than 20 ppm). If Sodium Hyaluronate is used in a parenteral preparation, heavy metals is not more than 10 ppm. Use 1.0 g of Sodium Hyaluronate for the test solution and 1.0 mL of standard lead solution, and proceed in the same manner as above.

(4) ***Iron***—Weigh accurately a portion of Sodium Hyaluronate, equivalent to 0.25 g of sodium hyaluronate on the dried bases, add 1 mL of nitric acid, heat to dissolve in a water-bath, cool, add water to make exactly 10 mL and use this solution as the test solution. Separately, pipet 1.0 mL and 2.0 mL of standard iron solution, prepare in the same manner as the test solution and use so obtained solutions as the standard solutions (1) and (2), respectively. Perform the test with the test solution, the standard solutions (1) and (2) as directed in the standard addition method under the Atomic Absorption Spectrophotometry (not more than 80 ppm).

Gas: Dissolved acetylene – Air
Lamp: An iron hollow cathode lamp
Wavelength: 248.3 nm

(5) ***Sulfated glycosaminoglycan***—When Sodium Hyaluronate is extracted from cocks' combs, it meets the requirement when the purity is tested according to the following method. Weigh a portion of Sodium Hyaluronate, equivalent to 50.0 mg of sodium hyaluronate on the dried basis, transfer to a test tube of 150 mm in length and 16 mm in internal diameter, dissolve in 1 mL of perchloric acid and use this solution as the test solution. Dissolve 0.149 g of sodium sulfate decahydrate and add water to make 100.0 mL. Pipet 10.0 mL of this solution and add water to make 100.0 mL. Transfer 1.0 mL of this solution to a fresh test tube of 150 mm in length and 16 mm in internal diameter, evaporate to dryness by heating to 90 to 95 °C, add 1.0 mL of perchloric acid to the residue to dissolve and use this solution as the standard solution. Plug each test tube with a piece of glass wool. Heat the test solution and the standard solution at 180 °C for 12 hours to obtain clear and colorless solution, and cool to room temperature. Add 3.0 mL each of 3.33 w/v % barium chloride solution, cap, shake vigorously and allow to stand for 30 minutes. Determine the absorption of these solutions at 660 nm, using water as blank, as directed under Ultraviolet-visible Spectrophotometry: the absorption from the test solution is not greater that that from the standard solution.

(6) ***Nucleic acids***—Dissolve a portion of Sodium Hyaluronate, equivalent to 0.1 g of sodium hyaluronate on the dried basis, in 30 mL of 0.9 w/v % sodium chloride solution by a gentle shaking for 12 hours and determine the absorbance of this solution at 260 nm as directed under Ultraviolet-visible Spectrophotometry: not more than 0.5.

(7) ***Protein***—Weigh accurately a portion of Sodium Hyaluronate, equivalent to 0.1 g of sodium hyaluronate on the dried bases, add water to make exactly 10 mL and use this solution as the test solution (1). Mix the test solution (1) with the equal volume of water and use this solution as the test solution (2). Weigh accurately 50 mg of bovine serum albumin and add water to make exactly 100 mL. Pipet 1.0, 3.0, 5.0, 7.0 and 10.0 mL of this solution, add water to make 100 mL each and use these solutions as the standard solutions. Pipet 2.5 mL each of water (blank), the test solution (1), the test solution (2) and the standard solutions, add 2.5 mL each of freshly prepared cupuric tartarate TS and mix for 10

minutes. Then, add 0.5 mL each of a mixture of water and phophomolybdotungstic TS (1:1), prepared immediately before use, and allow the tubes to stand for 30 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry and determine the absorbances at 750 nm, using water as the blank. Construct the calibration curve with the absorbances from the standard solutions and calculate the content of protein from the calibration curve: not more than 0.3 %, not more than 0.1 % for parenteral dosage forms.

Loss on Drying Not more than 20.0 % (0.50 g, 105 °C, 6 hours, P_2O_5).

Sterility Test It meets the requirement, when Sodium Hyaluronate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.5 EU/mg of Sodium Hyaluronate for parenteral preparations without a further procedure for the removal of bacterial endotoxins. Less than 0.05 EU/mg of Sodium Hyaluronate for intra-ocular preparations or intra-articular preparations without a further procedure for the removal of bacterial endotoxins.

Microbial Limit The total aerobic microbial count is not more than 100 CFU/g of Sodium Hyaluronate.

Assay Determine the content of glucuronic acid in the test specimen by the reaction with carbazole. Weigh accurately about 0.170 g of Sodium Hyaluronate, add water to make exactly 100 g, pipet 10 g of the solution, add water to make exactly 200 g and use this solution as the test solution. Repeat this procedure to obtain the test solution in three sets. Weigh accurately about 0.1 g of D-glucuronic acid, previous dried under vacuum to a constant mass, add water to make exactly 100 g. Pipet a portion of this solution, add water to prepare 5 solutions having the concentration between 6.5 and 65 μg of D-glucuronic acid per g water and use these solutions as the standard solutions. Prepare 25 test tubes, number the tubes 1 to 25, and place the tubes in iced water. Transfer 1.0 ml each of the five standard solutions in triplicate to the test tubes 1 to 15 (standard tubes), and 1.0 ml each of the three sets of the test solution in triplicate to the test-tubes 16 to 24 (test tubes). Transfer 1.0 mL of water to the test tube 25 (blank). Add 5.0 mL each of the freshly prepared 0.95 w/v % sodium tetraborate decahydrate in sulfuric acid solution to the test tubes. Tightly close the test-tubes with plastic caps, mix by shaking and place in a water-bath for exactly 15 min. Cool in iced water, and add 0.20 mL each of 0.125 w/v % carbazole in ethanol (95) to the test tubes. Recap the tubes, mix by shaking, and put them again in a water-bath for exactly 15 min. Cool to room temperature and measure the absorbances of the solutions at 530 nm against the blank. From the calibration curve obtained with the mean absorbances for each standard solution, determine the mean concentration of D-glucuronic acid in the test solutions.

$$\text{Content (\%) of sodium hyaluronate } (C_{14}H_{20}NNaO_{11})_n = \frac{C_g}{C_S} \times Z \times \frac{100}{100-h} \times \frac{401.3}{194.1}$$

C_g: Mean concentration (mg/g) of D-glucuronic acid in the test solutions

C_S: Mean concentration (mg/g) of Sodium Hyaluronate in the test solutions

Z: Content (%) of D-glucuronic acid ($C_6H_{10}O_7$)

h: Loss on drying (%)

401.3: Relative molecular mass of disaccharides

194.1: Relative molecular mass of glucuronic acid

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Sodium Iodide

NaI: 149.89

[*7681-82-5*]

Sodium Iodide, when dried, contains not less than 99.0 % and not more than 101.0 % of sodium iodide (NaI).

Description Sodium Iodide appears as colorless crystals or a white, crystalline powder and is odorless.
Sodium Iodide is very soluble in water and freely soluble in glycerin or in ethanol (95).
Sodium Iodide deliquesces in moist air.

Identification A solution of Sodium Iodide (1 in 20) responds to the Qualitative Tests for sodium salt and for iodide.

Purity (1) ***Clarity and color of solution***— Dissolve 1.0 g of Sodium Iodide in 2 mL of water: the solution is clear and colorless.

(2) ***Alkali***—Dissolve 1.0 g of Sodium Iodide in 10 mL of freshly boiled and cooled water and add 1.0 mL of 0.005 mol/L sulfuric acid VS and 1 drop of phenolphthalein TS: no color is observed.

(3) ***Chloride, bromide and thiosulfate***— Dissolve 0.20 g of Sodium Iodide in 5 mL of ammonia TS, add 15.0 mL of 0.1 mol/L silver nitrate VS, shake for a few minutes and filter. To 10 mL of the filtrate, add 15 mL of dilute nitric acid: no brown color is observed. The solution has no more turbidity than the following control solution.

Control solution–To 0.30 mL of 0.01 mol/L hydrochloric acid VS, add 2.5 mL of ammonia TS, 7.5 mL of 0.1 mol/L silver nitrate VS and 15 mL of dilute nitric acid.

(4) ***Nitrate, nitrite and ammonium***—Place 1.0 g of Sodium Iodide in a test tube and add 5 mL of water, 5 mL of sodium hydroxide TS and 0.2 g of aluminum wire. Insert a pledget of absorbent cotton in the mouth of the test tube and place a piece of moistened red litmus paper on the cotton. Heat the test tube on a water-bath for 15 minutes: the evolved gas does not turn moistened red litmus paper to blue.

(5) ***Cyanide***—Dissolve 0.5 g of Sodium Iodide in 10 mL of water. To 5 mL of this solution, add 1 drop of iron (II) sulfate TS and 2 mL of sodium hydroxide TS, warm and add 4 mL of hydrochloric acid: no green color is observed.

(6) ***Iodate***—Dissolve 0.5 g of Sodium Iodide in 10 mL of freshly boiled and cooled water and add 2 drops of dilute sulfuric acid and 1 drop of starch TS: no blue color is observed immediately.

(7) ***Heavy metals***—Proceed with 2.0 g of Sodium Iodide according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(8) ***Barium***—Dissolve 0.5 g of Sodium Iodide in 10 mL of water, add 1 mL of dilute sulfuric acid and allow to stand for 5 minutes: no turbidity is produced.

(9) ***Potassium***—Dissolve 1.0 g of Sodium Iodide in water and add water to make 100 mL. To 4.0 mL of this solution, add 1.0 mL of dilute acetic acid, shake, add 5.0 mL of a solution of sodium tetraphenylboron (1 in 30), immediately shake and allow to stand for 10 minutes: the solution has no more turbidity than the following control solution.

Control solution—Dissolve 9.5 mg of potassium chloride in water and add water to make 1000 mL. To 4.0 mL of this solution, add 1.0 mL of dilute acetic acid, shake and then proceed as directed above.

(10) ***Arsenic***— Prepare the test solution with 0.40 g of Sodium Iodide according to Method 1 and perform the test (not more than 5 ppm).

Loss on Drying Not more than 5.0 % (2 g, 120 °C, 2 hours).

Assay Weigh accurately about 0.4 g of Sodium Iodide, previously dried, in an iodine flask, dissolve in 10 mL of water, add 35 mL of hydrochloric acid and 5 mL of chloroform and titrate with 0.05 mol/L potassium iodate VS while shaking vigorously until the red-purple color of the chloroform layer disappears. The end point is attained only when the red-purple color does not reappear in the chloroform layer within 5 minutes after the layer has been decolorized.

Each mL of 0.05 mol/L potassium iodate VS
= 14.989 mg of NaI

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Sodium Iodide (^{123}I) Injection

Sodium Iodide (^{123}I) Injection contains ^{123}I as a form of Sodium Iodide.
Sodium Iodide (^{123}I) Injection contains not less than 90.0 % and not more than 110.0 % of the labeled of ^{123}I radioactivity at the date and hour stated on the label.

Preparation (1) Iodine-123 is obtained from proton irradiation of tellurium-124. Separate and prepare it as a form of Sodium Iodide (^{123}I). Sodium Iodide (^{123}I) Injection is produced following the preparation of Injections.

(2) Iodine-123 is obtained from by proton irradiation of xenon enriched in xenon-124 followed by the decay of xenon-123 which is formed directly and by the decay of caesium-123. Separate and prepare it as a form of Sodium Iodide (^{123}I). Sodium Iodide (^{123}I) Injection is produced following the preparation of Injections.

Description Sodium Iodide (^{123}I) Injection is a clear, colorless liquid and is odorless.

Identification (1) Place the ^{123}I standard gamma ray source at a certain distance from the gamma ray detector, and determine the gamma ray spectrum. Calculate the relationship between gamma ray energy and spectrum peaks produced by the photoelectric effect at suitable intervals from low energy to high energy, and plot an energy calibration curve. Determine the gamma ray spectrum of a suitable volume of Sodium Iodide (^{123}I) Injection: the test solution exhibits a maximum at 0.159 MeV.

(2) Perform the test as directed under the Purity (1): A maximum radioactivity is obtained from the principal spot, corresponding to iodine-123, in the radiochromatogram.

pH 7.0 ~ 9.0

Purity (1) ***Radiochemical impurities***—Perform the test with a suitable volume of Sodium Iodide (^{123}I) Injection as directed under Thin-layer Chromatography. Develop the plate with 75 % methanol to a distance of about 1 cm: the radioactivity of any impurities, apart from sodium iodide (^{123}I), is not more than 5 % of the total radioactivity.

(2) ***Radionuclidic purity***—Perform the test (1) in the Identification with a portion of Sodium Iodide (^{123}I) Injection, and determine the radioactivity of ^{123}I (0.159 MeV) and ^{124}I (0.603 MeV). The radioactivity of ^{123}I is not less than 95 % of the total radioactivity at the date and hour stated on the label.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 175/V EU/mL of Sodium Iodide (^{123}I) Injection, where V is the maximum recommended dose per mL during the effective time.

Assay Determine the ionization current or converted indicator value (hereinafter "ionization current value") of gamma rays emitted from the sample and RS using an ionization chamber under the same conditions, and compare the results. Weigh accurately a certain volume each of the sample and RS of known radioactivity, dilute each with a solvent if necessary, and use these solutions as the test solution and standard solution, respectively. Put the same volume of the test solution and standard solution into measuring vessels of the same material and shape, place the vessels at certain locations in the ionization chamber, determine the ionization current value of each, and calculate the radioactivity of a certain volume of the sample by the following equation.

$$\text{Radioactivity of a certain amount of the sample} = S \times \frac{I}{I'} \times \frac{D}{D'} \times G$$

S: Radioactivity of a certain volume of the RS
I: Ionization current value of the test solution
I': Ionization current value of the standard solution
D: Dilution factor of the sample
D': Dilution factor of the RS
G: Correction term including the geometric conditions of the positions of the test solution and standard solution
Where possible, G = 1.

Expiration Date Within 48 hours from the date and hour stated on the label.

Sodium Iodide (^{131}I) Capsules

Sodium Iodide (^{131}I) Capsules contain iodine (^{131}I) as a form of Sodium Iodide.
Sodium Iodide (^{131}I) Capsules conform to the requirements of Sodium Iodide (^{131}I) Capsules in the Korean Pharmaceutical Codex.

Sodium Iodide (^{131}I) Solution

Sodium Iodide (^{131}I) Solution contains iodine-131 (^{131}I) in the form of Sodium Iodide.
Sodium Iodide (^{131}I) Solution conforms to the requirements of Sodium Iodide (^{131}I) Solution in the Korean Pharmaceutical Codex.

Description Sodium Iodide (^{131}I) Solution is a clear, colorless liquid. Sodium Iodide (^{131}I) Solution is odorless or has an odor of the preservative or stabilizer.

Sodium Iodohippurate (^{131}I) Injection

Sodium Iodohippurate (^{131}I) Injection is an aqueous solution for injection containing iodine-131 (^{131}I) in the form of sodium *o*-iodohippurate.
Sodium Iodohippurate (^{131}I) Injection conforms to the requirements of Sodium Iodohippurate (^{131}I) Injection in the Korean Pharmaceuticals Codex.
The Insoluble Particulate Matter Test for Injections is not applied to this injection.

Description Sodium Iodohippurate (^{131}I) Injection is a clear and colorless liquid and is odorless or has an odor of the preservative or stabilizer.

Bacterial Endotoxins Less than 175/V EU/mL of Sodium Iodohippurate (^{131}I) Injection, where V is the maximum recommended dose per mL during the effective time.

Sodium Pertechnetate (^{99m}Tc) Injection

Sodium Pertechnetate (^{99m}Tc) Injection is an aqueous solution for injection containing technetium-99m (^{99m}Tc) in the form of sodium pertechnetate.
Sodium Pertechnetate Injection conforms to the requirements of Sodium Pertechnetate (^{99m}Tc) Injection in the Korean Pharmaceutical Codex.

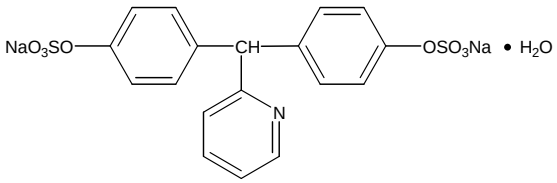
Description Sodium Pertechnetate (^{99m}Tc) Injection is a clear, colorless liquid.

Sodium Phosphate (^{32}P) Solution

Sodium Phosphate (^{32}P) Solution contains Phosphorous-32 in the form of sodium phosphate.
Sodium Phosphate (^{32}P) Solution conforms to the requirements of Sodium Phosphate (^{32}P) Solution in the Korean Pharmaceutical Codex.

Description Sodium Phosphate (^{32}P) Solution is a clear, colorless liquid.

Sodium Picosulfate Hydrate



Sodium Picosulfate $C_{18}H_{13}NNa_2O_8S_2 \cdot H_2O$: 499.42

Sodium 4,4'-(pyridin-2-ylmethylene)bis(4,1-phenylene) disulfate [*10040-45-6,* anhydride]

Sodium Picosulfate Hydrate contains not less than 98.5 % and not more than 101.0 % of sodium picosulfate hydrate ($C_{18}H_{13}NNa_2O_8S_2 \cdot H_2O$: 481.41), calculated on the anhydrous basis.

Description Sodium Picosulfate Hydrate is a white crystalline powder, is odorless and tasteless.
Sodium Picosulfate Hydrate is very soluble in water, soluble in methanol, slightly soluble in ethanol (99.5) and practically insoluble in ether.
Sodium Picosulfate Hydrate is gradually colored by light.

pH—Dissolve 1.0 g of Sodium Picosulfate in 20 mL of water: the pH of this solution is between 7.4 and 9.4.

Identification (1) To 5 mg of Sodium Picosulfate Hydrate, add 10 mg of 1-chloro-2,4-dinitrobenzene, mix and fuse by gentle heating for 5 to 6 seconds. After cooling, add 4 mL of potassium hydroxide and ethanol TS: an orange color is observed.

(2) To 0.2 g of Sodium Picosulfate Hydrate, add 5 mL of dilute hydrochloric acid, boil for 5 minutes, cool and add 1 mL of barium chloride TS: a white precipitate is produced.

(3) Determine the absorbance spectra of the aqueous solutions of Sodium Picosulfate Hydrate and Sodium Picosulfate Hydrate RS (1 in 25000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared spectra of Sodium Picosulfate Hydrate and Sodium Picosulfate Hydrate RS, previously dried at 105 ºC in vaccum for 4 hour, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(5) A solution of Sodium Picosulfate Hydrate (1 in 10) responds to the Qualitative Tests for sodium salt.

Absorbance $E^{1\%}_{1cm}$ (263 nm): 120 ~ 130 (4 mg, calculated on the anhydrous basis, water, 100 mL).

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Sodium Picosulfate Hydrate in 10 mL of water: the solution is colorless to pale yellow.

(2) ***Chloride***—Weigh 0.5 g of Sodium Picosulfate Hydrate and perform the test. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid (not more than 0.028 %).

(3) ***Sulfate***—Weigh 0.40 g of Sodium Picosulfate Hydrate and test. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid (not more than 0.042 %).

(4) ***Heavy metals***—Proceed with 2.0 g of Sodium Picosulfate Hydrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) ***Arsenic***—Prepare the test solution with 2.0 g of Sodium Picosulfate Hydrate according to Method 3 and perform the test (not more than 1 ppm).

(6) ***Related substances***—Dissolve 0.25 g of Sodium Picosulfate Hydrate in 5 mL of methanol and use this solution as the test solution. Pipet 1 mL of the test solution, add methanol to make exactly 500 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the chromatogram with a mixture of n-butanol, water and acetic acid (100) (74 : 20 : 19) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (principal wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Water 3.0 ~ 4.5 % (0.5 g, volumetric titration, direct titration).

Assay Dissolve about 0.4 g of Sodium Picosulfate Hydrate, accurately weighed, in 50 mL of methanol, add 7 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 48.14 mg of $C_{18}H_{13}NNa_2O_8S_2$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Sodium Polystyrene Sulfonate

Sodium poly(2-ethenylbenzenesulfonate) [*178955-71-0*]

Sodium Polystyrene Sulfonate is a cation exchange resin prepared as the sodium form of the sulfonated styrene divinylbenzene copolymer.
Sodium Polystyrene Sulfonate contains not less than 9.4 % and not more than 11.0 % of sodium (Na: 22.99),

calculated on the anhydrous basis.
Each g of Sodium Polystyrene Sulfonate, calculated on the anhydrous basis, exchanges with 0.110 to 0.135 g of potassium (K: 39.10).

Description Sodium Polystyrene Sulfonate appears as yellow-brown powder, and is odorless and tasteless.
Sodium Polystyrene Sulfonate is practically insoluble in water, in ethanol (95), in acetone or in ether.

Identification (1) Determine the infrared spectra of Sodium Polystyrene Sulfonate and Sodium Polystyrene Sulfonate RS as directed in the potassium bromide disk method the Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) To 1.0 g of Sodium Polystyrene Sulfonate, add 10 mL of dilute hydrochloric acid, mix with swirling, filter and neutralize the filtrate with ammonia TS: the solution responds to the Qualitative Tests for sodium salt.

Purity (1) ***Ammonia***—Place 1.0 g of Sodium Polystyrene Sulfonate in a flask, add 5 mL of sodium hydroxide TS, cover the flask with a watch glass having a moistened strip of red litmus paper on the underside and boil for 15 minutes: the gas evolved does not change the red litmus paper to blue.

(2) ***Heavy metals***—Proceed with 2.0 g of Sodium Polystyrene Sulfonate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Prepare the test solution with 2.0 g of Sodium Polystyrene Sulfonate according to Method 3 and perform the test (not more than 1 ppm).

(4) ***Styrene***—To 10.0 g of Sodium Polystyrene Sulfonate, add 10 mL of acetone, shake for 30 minutes, centrifuge and use the clear supernatant liquid as the test solution. Separately, dissolve 10.0 mg of styrene in acetone to make exactly 100 mL. Pipet 1.0 mL of this solution, dilute with acetone to make exactly 100 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine the peak heights, A_T and A_S, of styrene for the test solution and the standard solution, respectively: A_T is not larger than A_S.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of water and acetonitrile (1 : 1).

Flow rate: Adjust the flow rate so that the retention time of styrene is about 7 to 8 minutes.

System suitability

System performance: Dissolve 20 mg each of styrene and butyl parahydroxybenzoate in 100 mL of acetone. Pipet 5 mL of this solution and add acetone to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, butyl parahydroxybenzoate and styrene are eluted in this order with the resolution between these peaks being not less than 5.0.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of styrene is not more than 2.0 %.

Water Not more than 10.0 % (0.2 g, volumetric titration, direct titration).

Assay (1) ***Sodium***—Pipet 50 mL of 3 mol/L hydrochloric acid TS into a glass-stoppered flask containing about 1.0 g of Sodium Polystyrene Sulfonate calculated on the anhydrous basis, accurately weighed, stir for 60 minutes and filter. Discard the first 20 mL of the filtrate, pipet 5.0 mL of the subsequent filtrate and add water to make exactly 100 mL, pipet 20.0 mL of this solution, add water to make exactly 1000 mL and use this solution as the test solution. Separately, measure exactly a suitable volume of standard sodium chloride stock solution, dilute with water to make solutions containing 1 g to 3 g of sodium (Na: 22.99) per mL and use these solutions as the standard solutions. Perform the test with the test solution and the standard solutions as directed under the Atomic Absorption Spectrophotometry according to the following conditions and determine the amount of sodium in the test solution, using the calibration curve obtained from the standard solutions.

Gas used: Dissolved acetylene – Air
Lamp: A sodium hollow-cathode lamp.
Wavelength: 589.0 nm.

(2) ***Potassium exchange capacity***—Pipet 100.0 mL of standard potassium stock solution into a glass-stoppered flask containing about 1.5 g of Sodium Polystyrene Sulfonate calculated on the anhydrous basis, accurately weighed, stir for 15 minutes and filter. Discard the first 20 mL of the filtrate, pipet 10.0 mL of the subsequent filtrate and add water to make exactly 100 mL. Pipet 10.0 mL of this solution, add water to make exactly 1000 mL and use this solution as the test solution. Separately, measure a suitable volume of standard potassium stock solution, dilute with water to make solutions containing 1 μg to 5 μg of potassium (K: 39.10) per mL and use these solutions as the standard solutions. Perform the test with the test solution and the standard solutions as directed under the Atomic Absorption Spectrophotometry according to the following conditions and determine the amount, Y (mg), of potassium in 1000 mL of the test solution, using the calibration curve obtained from the standard solutions. The

exchange quantity for potassium per g of Sodium Polystyrene Sulfonate, calculated on the anhydrous basis, is calculated by the following equation.

$$\text{Exchange quantity (mg) for potassium (K) Per g of Sodium Polystyrene Sulfonate, calculated on the anhydrous basis} = \frac{X - 100Y}{W}$$

X: Amount (mg) of potassium in 100 mL of standard potassium stock solution before exchange,
W: Amount (g) of Calcium Polystyrene Sulfonate taken, calculated on the anhydrous basis.

Gas used: Dissolved acetylene – Air
Lamp: A potassium hollow-cathode lamp.
Wavelength: 766.5 nm.

Containers and Storage ***Containers***—Tight containers.

Sodium Pyrosulfite

Sodium Metabisulfite $Na_2S_2O_5$: 190.11

Disodiumpyrosulfite [*7681-57-4*]

Sodium Pyrosulfite contains not less than 95.0 % and not more than 101.0 % of sodium pyrosulfite ($Na_2S_2O_5$).

Description Sodium Pyrosulfite appears as white crystals or crystalline powder and has the odor of sulfur dioxide.
Sodium Pyrosulfite is freely soluble in water, very slightly soluble in ethanol (95), and practically insoluble in ether.
A solution of Sodium Pyrosulfite (1 in 20) is acidic.
Sodium Pyrosulfite is hygroscopic.
Sodium Pyrosulfite decomposes gradually on exposure to air.

Identification A solution of Sodium Pyrosulfite (1 in 20) responds to the Qualitative Tests for sodium salt and for bisulfite.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Sodium Pyrosulfite in 10 mL of water: the solution is clear and colorless.

(2) ***Thiosulfate***—Dissolve 1.0 g of Sodium Pyrosulfite in 15 mL of water, add slowly 5 mL of dilute hydrochloric acid, shake and allow to stand for 5 minutes: no turbidity is produced.

(3) ***Heavy metals***—Dissolve 1.0 g of Sodium Pyrosulfite in 10 mL of water and evaporate with 5 mL of hydrochloric acid on a water-bath to dryness. Dissolve the residue in 10 mL of water, add 1 drop of phenolphthalein TS and add ammonia TS until the solution becomes slightly red. Add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 5 mL of hydrochloric acid on a water-bath to dryness and to the residue, add 2 mL of dilute acetic acid, 2.0 mL of standard lead solution and water to make 50 mL (not more than 20 ppm).

(4) ***Iron***—Prepare the test solution with 1.0 g of Sodium Pyrosulfite according to Method 1 and perform the test according to Method A. Prepare the control solution with 2.0 mL of standard iron solution (not more than 20 ppm).

(5) ***Arsenic***—Dissolve 0.5 g of Sodium Pyrosulfite in 10 mL of water, heat with 1 mL of sulfuric acid in a sand-bath until white fumes are evolved and add water to make 5 mL. Perform the test with this solution as the test solution (not more than 4 ppm).

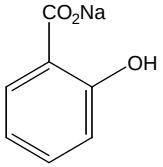
Assay Weigh accurately about 0.15 g of Sodium Pyrosulfite and transfer to an iodine flask containing an exactly measured 50 mL of 0.05 mol/L iodine VS. Stopper tightly, shake well and allow to stand for 5 minutes in a dark place. Add 1 mL of hydrochloric acid VS and titrate the excess of iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination and make any necessary correction.

Each mL of 0.05 mol/L iodine VS
= 4.753 mg of $Na_2S_2O_5$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, preferably well-filled, and not exceeding 30 °C.

Sodium Salicylate



$C_7H_5NaO_3$: 160.10

Sodium 2-hydroxybenzoate [*54-21-7*]

Sodium Salicylate, when dried, contains not less than 98.0 % and not more than 102.0 % of sodium salicylate ($C_7H_5NaO_3$).

Description Sodium Salicylate appears as white crystals or crystalline powder.
Sodium Salicylate is very soluble in water, freely soluble in acetic acid (100) and soluble in ethanol (95).
Sodium Salicylate is gradually colored by light.

Identification (1) Determine the infrared spectra of

Sodium Salicylate and Sodium Salicylate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absoption at the same wavenumbers.

(2) A solution of Sodium Salicylate (1 in 20) responds to the Qualitative Tests for sodium salt.

pH Dissolve 2.0 g of Sodium Salicylate in 20 mL of water: the pH of this solution is between 6.0 and 8.0.

Purify (1) ***Clarity and color of solution***—Dissolve 1.0 g of Sodium Salicylate in 10 mL of water: the solution is colorless. Perform the test as directed under Ultraviolet-visible Spectrophotometry and determine the absorbance of this solution at 420 nm: the absorbance is not more than 0.02.

(2) ***Chloride***—Dissolve 0.5 g of Sodium Salicylate in 15 mL of water, add 6 mL of dilute nitric acid and ethanol (95) to make 50 mL and perform the test. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS, 28 mL of ethanol (95), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.021 %).

(3) ***Sulfate***—Dissolve 0.25 g of Sodium Salicylate in 5 mL of water and add 0.5 mL of barium chloride TS: the solution shows no change.

(4) ***Sulfite and thiosulfate***—Dissolve 1.0 g of Sodium Salicylate in 20 mL of water, add 1 mL of hydrochloric acid and filter. Add 0.15 mL of 0.05 mol/L iodine VS to the filtrate: a yellow color develops.

(5) ***Heavy metals***—Proceed with 1.0 g of Sodium Salicylate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(6) ***Arsenic***—Place 1.0 g of Sodium Salicylate in decomposition flask, add 5 mL of nitric acid and 2 mL of sulfuric acid and heat cautiously until white fumes are evolved. After cooling, add 2 mL of nitric acid, and heat. After cooling, add several 2 mL portions of hydrogen peroxide (30) and heat again until the solution is colorless to pale yellow. Repeat the procedure of adding nitric acid and hydrogen peroxide (30) and heating, if neccessary. After cooling, add 2 mL of a saturated solution of ammonium oxalate and heat until white fumes are evolved again. After cooling, add water to make 5 mL and perform the test with this solution as the test solution (not more than 2 ppm).

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 2 hours).

Assay Weigh accurately each about 0.2 g of Sodium Salicylate and Sodium Salicylate RS, previously dried, dissolve in water to make exactly 100 mL. Pipet 10 mL each of these solutions, add 10.0 mL of the internal standard and water to make exactly 100 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 20 μL each of these solutions as directed under Liquid Chromatography under the following conditions and calculate the ratios, Q_T and Q_S, the peak area of sodium salicylate to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of sodium salicylate } (C_7H_5NaO_3) = \text{Amount (mg) of Sodium Salicylate RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—Weigh about 20 mg of Anhydrous Caffeine RS and dissolve in water to make 100 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 15 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Mobile phase: A mixture of water, methanol and acetic acid (31) (50 : 50 : 1).

Flow rate: Adjust the flow rate so that the retention time of sodium salicylate is about 8.5 minutes.

Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions. Use as column fiving elution of the internal standard and sodium salicylate in this order with the resolution between their peaks being no less than 4.0.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Sodium Thiosulfate Hydrate

$Na_2S_2O_3 \cdot 5H_2O$: 248.18

[*7772-98-7*, anhydride]

Sodium Thiosulfate Hydrate, when dried, contains not less than 99.0 % and not more than 101.0 % of sodium thiosulfate ($Na_2S_2O_3$: 158.11).

Description Sodium Thiosulfate Hydrate appears as colorless crystals or crystalline powder and is odorless.
Sodium Thiosulfate Hydrate is very soluble in water and very slightly soluble in ethanol (95) and practically insoluble in ether.
Sodium Thiosulfate Hydrate effervesces in dry air and is deliquescent in moist air.

Identification A solution of Sodium Thiosulfate Hydrate (1 in 10) responds to the Qualitative Tests for sodium salt and for thiosulfate.

pH Dissolve 1.0 g of Sodium Thiosulfate in 10 mL of water: the pH of the solution is between 6.0 and 8.0.

Purity (1) ***Clarity and color of solution*—**Dissolve 1.0 g of Sodium Thiosulfate Hydrate in 10 mL of water: the solution is clear and colorless.

(2) ***Heavy metals*—**Dissolve 1.0 g of Sodium Thiosulfate Hydrate in 10 mL of water, add slowly 5 mL of dilute hydrochloric acid and evaporate in a water-bath to dryness. Add 15 mL of water to the residue, boil gently for 2 minutes and filter. Heat the filtrate to boil and add bromine TS to the hot filtrate to produce a clear solution and provide a slight excess of bromine. Boil the solution to expel the bromine. After cooling, add 1 drop of phenolphthalein TS and add drop-wise sodium hydroxide TS until a slight red color is produced. Add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test. Prepare the control solution as follows: to 2.0 mL of standard lead solution, add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(3) ***Calcium*—**Dissolve 1.0 g of Sodium Thiosulfate Hydrate in 10 mL of water, add 2 mL of ammonium oxalate TS and allow to stand for 4 minutes: no turbidity is produced.

(4) ***Arsenic*—**To 0.40 g of Sodium Thiosulfate Hydrate, add 3 mL of nitric acid and 5 mL of water, evaporate in a water-bath to dryness and perform the test with the residue. Prepare the test solution according to Method 2 and perform the test (not more than 5 ppm).

Loss on Drying 32.0 ~ 37.0 % (1 g, in vacuum, 40 ~ 45 °C, 16 hours).

Assay Weigh accurately 0.4 g of Sodium Thiosulfate Hydrate, previously dried, dissolve in 30 mL of water and titrate with 0.05 mol/L iodine VS (indicator: 1 mL of starch TS).

Each mL of 0.05 mol/L iodine VS
= 15.811 mg of $Na_2S_2O_3$.

Containers and Storage ***Containers***—Tight containers.

Sodium Thiosulfate Injection

Sodium Thiosulfate Injection is an aqueous solution for injection. Sodium Thiosulfate Injection contains not less that 95.0 % and not more than 105.0 % of the labeled amount of sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$: 248.18).

Method of Preparation Prepare as directed under Injections, with Sodium Thiosulfate Hydrate.

Description Sodium Thiosulfate Injection is a clear, colorless liquid.

Identification Sodium Thiosulfate Injection responds to the Qualitative Tests for sodium salt and for thiosulfate.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.01 EU/mg of sodium thiosulfate.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

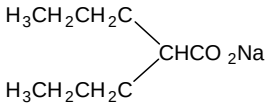
Determination of Volume of Injection in Containers It meets the requirement.

Assay Measure exactly a volume of Sodium Thiosulfate Injection, equivalent to about 0.5 g of sodium thiosulfate hydrate ($Na_2S_2O_3 \cdot 5H_2O$), add water to make 30 mL and titrate with 0.05 mol/L iodine VS (indicator: 1 mL of starch TS).

Each mL of 0.05 mol/L iodine VS
= 24.818 mg of $Na_2S_2O_3 \cdot 5H_2O$

Containers and Storage ***Containers***—Hermetic containers.

Sodium Valproate



$C_8H_{15}NaO_2$: 166.19

Sodium 2-propylpentanoate [*1069-66-5*]

Sodium Valproate, when dried, contains not less than 98.5 % and not more than 101.0 % of Sodium valproate ($C_8H_{15}NaO_2$).

Description Sodium Valproate is a white, crystalline powder, has a characteristic odor and a slightly bitter taste.
Sodium Valproate is very soluble in water, freely soluble in formic acid, in ethanol (95), in ethanol (99.5) or in acetic acid (100) and practically insoluble in chloroform or in ether.
Sodium Valproate is hygroscopic.

Identification (1) To 1 mL of a solution of Sodium Valproate in ethanol (99.5) (1 in 200), add 4 mL of hydroxylamine perchlorate-ethanol (99.5) TS and 1 mL of *N,N'*-dicyclohexyl-carbodiimide-ethanol (99.5) TS, shake well and allow to stand in lukewarm water for 20 minutes. After cooling, add 1 mL of iron (III) perchlorate hexahydrate-ethanol (99.5) TS and shake: a purple color is observed.

(2) To 5 mL of a solution of Sodium Valproate (1 in 20), add 1 mL of a solution of cobaltous nitrate (1 in 20) and warm in a water-bath: a purple precipitate is formed.

(3) Dissolve 0.5 g of Sodium Valproate in 5 mL of water, add 5 mL of chloroform and 1 mL of 2 mol/L hydrochloric acid TS and shake vigorously for 1 minute. Proceed the same procedure with Sodium Valproate RS. After allowing to stand, separate the chloroform layers, dehydrate the chloroform layers with anhydrous sodium sulfate and filter. Evaporate the solvent of the filtrates and determine the infrared spectra of the residues as directed in the liquid film method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(4) A solution of Sodium Valproate (1 in 10) responds to the Qualitative Tests for sodium salt.

pH Dissolve 1.0 g of Sodium Valproate in 20 mL of water: the pH of this solution is between 7.0 and 8.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Sodium Valproate in 10 mL of water: the solution is clear and colorless.

(2) ***Chloride***—Dissolve 0.5 g of Sodium Valproate in 25 mL of ethanol (95) and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test. Prepare the control solution as follows: to 0.70 mL of 0.01 mol/ L hydrochloric acid VS, add 25 mL of ethanol (95), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.050 %).

(3) ***Sulfate***—Dissolve 0.5 g of Sodium Valproate in 25 mL of ethanol (95) and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test. Prepare the control solution as follows: to 0.50 mL of 0.005 mol/L sulfuric acid VS, add 25 mL of ethanol (95), 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048 %).

(4) ***Heavy metals***—Dissolve 2.0 g of Sodium Valproate in 44 mL of water, shake with 6 mL of dilute hydrochloric acid, allow to stand for 5 minutes and filter. Discard the first 5 mL of the filtrate, neutralize the subsequent 25 mL with ammonia TS and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test. Prepare the control solution as follows: to 2.0 mL of standard lead solution, add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(5) ***Arsenic***—Dissolve 2.0 g of Sodium Valproate in 10 mL of water, shake with 10 mL of dilute hydrochloric acid, allow to stand for 5 minutes and filter. Discard the first 5 mL of the filtrate and perform the test with the subsequent 10 mL as the test solution (not more than 2 ppm).

(6) ***Related substances***—Dissolve 0.10 g of Sodium Valproate in 10 mL of a mixture of formic acid and chloroform (1 : 1) and use this solution as the test solution. Pipet 1.0 mL of the test solution, add a mixture of formic acid and chloroform (1:1) to make exactly 200 mL and use this solution as the standard solution. Perform the test with 2 μL each of the test solution and the standard solution as directed under Gas Chromatography according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of all peaks other than the area of the valproic acid from the test solution is not larger than the peak area of the valproic acid from the standard solution.

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A column, about 3 mm in internal diameter and about 2 m in length, packed with siliceous earth for gas chromatography (150 μm to 180 μm in particle diameter) coated with diethylene glycol adipate ester for gas chromatography and phosphoric acid at the ratios of 5 % and 1 %, respectively.

Column temperature: A constant temperature of about 145 °C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of valproic acid is between 6 minutes and 10 minutes.

System suitability

Detection sensitivity: Adjust the detection sensitivity so that the peak height of valproic acid obtained from 2 μL of the standard solution is between 4 mm and 10 mm.

System performance: Mix 1 mL of the test solution and 4 mL of a solution of n-valerianic acid in a mixture of formic acid and chloroform (1 : 1) (1 in 1000). When the procedure is run with 2 μL of this solution under the above operating conditions, n-valerianic acid and valproic acid are eluted in this order with a resolution between their peaks being not less than 3.

Time span of measurement: About twice as long as the retention time of valproic acid after the solvent peak.

Loss on Drying Not more than 1.0 % (1 g, 105 °C, 3 hours).

Assay Weigh accurately about 0.2 g of Sodium Valproate, previously dried, dissolve in 80 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 16.619 mg of $C_8H_{15}NaO_2$

Containers and Storage ***Containers***—Tight containers.

D-Sorbitol Solution

D-Sorbitol Solution Contains not less than 97.0 % and not more than 103.0 % of the labeled amount of D-sorbitol ($C_6H_{14}O_6$: 182.17).

Description D-Sorbitol Solution is a clear, colorless liquid, is odorless and has a sweet taste.
D-Sorbitol is miscible with water, with ethanol (95), with glycerin or with propylene glycol.
D-Sorbitol Solution sometimes separates crystalline masses.

Identification (1) Take a volume of D-Sorbitol Solution, equivalent to 0.7 g of D-Sorbitol according to the labeled amount, proceed as directed in the Identification (1) under D-Sorbitol.

(2) Take a volume of D-Sorbitol Solution, equivalent to 1 g of D-Sorbitol according to the labeled amount, add water to make 20 mL. Pipet 1 mL of this solution, proceed as directed in the identification (2) under D-Sorbitol.

Purity (1) ***Acidity or alkalinity***—D-Sorbitol Solution is neutral.

(2) ***Chloride***—Proceed with a volume of D-Sorbitol Solution, equivalent to 2.0 g of D-Sorbitol according to the labeled amount, proceed as directed in the Purity (2) under D-Sorbitol (not more than 0.005 %).

(3) ***Sulfate***—Take a volume of D-Sorbitol Solution, equivalent to 4.0 g of D-Sorbitol according to the labeled amount, proceed as directed in the Purity (3) under D-Sorbitol (not more than 0.006 %).

(4) ***Heavy metals***—Proceed with a volume of D-Sorbitol Solution, equivalent to 5.0 g of D-Sorbitol according to the labeled amount, proceed as directed in the Purity (4) under D-Sorbitol (not more than 5 ppm).

(5) ***Nickel***—Take a volume of D-Sorbitol Solution, equivalent to 0.5 g of D-Sorbitol according to the labeled amount, proceed as directed in the Purity (5) under D-Sorbitol.

(6) ***Arsenic***—Take a volume of D-Sorbitol Solution, equivalent to 1.5 g of D-Sorbitol according to the labeled amount, dilute with water or concentrate to 5 mL on a water-bath, if necessary, cool and perform the test using this solution as the test solution (not more than 1.3 ppm).

(7) ***Glucose***—Take a volume of D-Sorbitol Solution, equivalent to 20.0 g of D-Sorbitol according to the labeled amount, dilute with water or concentrate to 40 mL on a water-bath, if necessary, add 40 mL of Fehling's TS and proceed as directed in the Purity (7) under D-Sorbitol.

(8) ***Sugars***—Take a volume of D-Sorbitol Solution, equivalent to 20.0 g of D-Sorbitol according to the labeled amount, proceed as directed in the Purity (8) under D-Sorbitol.

(9) ***Ethylene glycol and diethylene glycol***—Weigh accurately about 2.0 g of D-Sorbitol Solution, transfer to a 25 mL volumetric flask, add 1 mL of the diluent, and shake for about 3 minutes. Add the remaining diluent in three volumes to make exactly 25 mL, while shaking for about 3 minutes with each addition. Take the clear supernatant liquid, filter through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 2 mL of the filtrate, and use the subsequent filtrate as the test solution. Separately, dissolve a suitable amount each of Diethylene Glycol RS and Ethylene Glycol RS in the diluent so that each mL contains 0.08 mg of each, and use this solution as the standard solution. Perform the test with 1.0 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the peak area of diethylene glycol from the test solution is not larger than the peak area of diethylene glycol from the standard solution (not more than 0.10 %), and the peak area of ethylene glycol from the test solution is not larger than the peak area of ethylene glycol from the standard solution (not more than 0.10 %).

Operating conditions

Detector: A hydrogen flame-ionization detector

Column: A quartz glass column about 0.32 mm in internal diameter and about 15 m in length, the inside coated with cyanopropylphenyl-dimethylpolysiloxane for gas chromatography (6 : 94) 0.25 μm in thickness.

Column temperature: Maintain at 70 °C for 2 minutes, then increase the temperature to 300 °C at the rate of 50 °C per minute, and maintain at 300 °C for 5 minutes.

Injection port temperature: A constant temperature of about 240 °C

Detector temperature: A constant temperature of about 300 °C

Carrier gas: Helium

Flow rate: 3.0 mL/minute

Split ratio: About 1 : 10

System suitability

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, ethylene glycol and diethylene glycol are eluted in this order with the resolution between these peaks being not less than 30.

Diluent—A mixture of acetone and water (96 : 4)

Residue on Ignition Take exactly a volume of D-Sorbitol Solution, equivalent to 5 g of D-Sorbitol according to the labeled amount, add 3 to 4 drops of sulfuric acid and heat gently to evaporate. Ignite to burn, cool and perform the test with the residue: not more than 1.0 mg.

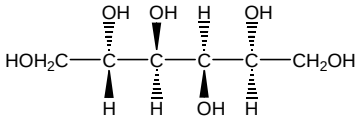
Assay Take exactly a volume of D-Sorbitol Solution, equivalent to about 5 g of D-sorbitol ($C_6H_{14}O_6$) according to the labeled amount and add water to make exactly 250 mL. Pipet 10 mL of this solution, add water to

make exactly 100 mL. Pipet 10 mL of the solution into an iodine flask and proceed as directed in the Assay under D-Sorbitol.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 1.8217 mg of $C_6H_{14}O_6$

Containers and Storage ***Containers*****—**Tight containers.

D-Sorbitol



$C_6H_{14}O_6$: 182.17

(2*R*,3*R*,4*R*,5*S*)-Hexane-1,2,3,4,5,6-hexol [*50-70-4*]

D-Sorbitol, when dried, contains not less than 97.0 % and not more than 101.0 % of D-sorbitol ($C_6H_{14}O_6$).

Description D-Sorbitol is a white granule, powder, or crystalline mass, is odorless and has a sweet taste with a cold sensation.
D-Sorbitol is very soluble in water, sparingly soluble in ethanol (95) and practically insoluble in ether.
D-Sorbitol is hygroscopic.

Identification (1) To 1 mL of a solution of D-Sorbitol (7 in 10), add 2 mL of iron (II) sulfate TS and 1 mL of a solution of sodium hydroxide (1 in 5): a blue-green color is observed, but no turbidity is produced.

(2) Shake thoroughly 1 mL of a solution of D-Sorbitol (1 in 20) with 1 mL of a freshly prepared solution of catechol (1 in 10), add rapidly 2 mL of sulfuric acid and shake: a reddish purple to red-purple color immediately is observed.

(3) Boil 0.5 g of D-Sorbitol with 10 mL of acetic anhydride and 1 mL of pyridine under a reflux condenser for 10 minutes, cool, shake with 25 mL of water and allow to stand in a cold place. Transfer the solution to a separatory funnel, extract with 30 mL of chloroform and evaporate the extract on a water-bath. Add 80 mL of water to the oily residue, heat for 10 minutes in a water-bath, then filter the hot mixture. After cooling, collect the produced precipitate through a glass filter (G3), wash with water, recrystallize once from ethanol (95) and dry in a desiccator (in vacuum, silica gel) for 4 hours: the precipitate melts between 97 °C and 101 °C.

Purity (1) ***Clarity and color of solution and acidity or alkalinity***—Dissolve 5 g of D-Sorbitol in 20 mL of water by warming with shaking: the solution is clear, colorless and neutral.

(2) ***Chloride***—Perform the test with 2.0 g of D-Sorbitol. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.005 %).

(3) ***Sulfate***—Perform the test with 4.0 g of D-Sorbitol. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.006 %).

(4) ***Heavy metals***—Proceed with 5.0 g of D-Sorbitol according to Method 1 and perform the test. Prepare the control solution with 2.5 mL of standard lead solution (not more than 5 ppm).

(5) ***Nickel***—Dissolve 0.5 g of D-Sorbitol in 5 mL of water, add 3 drops of dimethylglyoxime TS and 3 drops of ammonia TS and allow to stand for 5 minutes: no red color develops.

(6) ***Arsenic***—Prepare the test solution with 1.5 g of D-Sorbitol according to Method 1 and perform the test (not more than 1.3 ppm).

(7) ***Glucose***—Dissolve 20.0 g of D-Sorbitol in 25 mL of water and boil gently with 40 mL of Fehling's TS for 3 minutes, After cooling, filter the clear supernatant liquid cautiously through a glass filter (G4), leaving the precipitate in the flask as much as possible, wash the precipitate with hot water until the last washings no longer show an alkali reaction and filter the washings through the glass filter. Dissolve the precipitate in 20 mL of iron (II) sulfate TS in a flask, filter through the glass filter and wash with water. Combine the filtrate and the washings, heat at 80 °C and titrate with 0.02 mol/L potassium permanganate VS: not more than 6.3 mL is consumed.

(8) ***Sugars***—Dissolve 20.0 g of D-Sorbitol in 25 mL of water and heat with 8 mL of dilute hydrochloric acid under a reflux condenser in a water-bath for 3 hours. After cooling, add 2 drops of methyl orange TS, followed by sodium hydroxide TS until an orange color develops and add water to make 100 mL. Boil gently 10 mL of this solution with 10 mL of water and 40 mL of Fehling's TS for 3 minutes and proceed as directed in (7).

Loss on Drying Not more than 2.0 % (0.5 g, in vacuum, P_2O_5, 80 °C, 3 hours).

Residue on Ignition Not more than 0.02 % (5 g),

Microbial Limit The total aerobic microbial count is not more than 1000 CFU/g, the total combined yeasts/mould count is not more than 100 CFU/g and *Escherichia coli* and *Salmonella* are not observed.

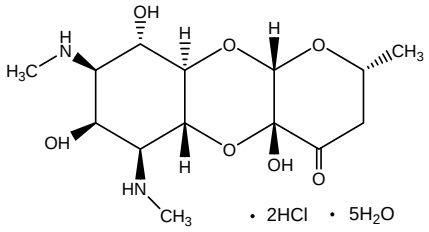
Assay Weigh accurately about 0.2 g of D-Sorbitol, previously dried, dissolve in water and add water to make exactly 100 mL. Pipet 10 mL of this solution into an iodine flask, add exactly 50 mL of potassium periodate TS and heat for 15 minutes in a water-bath. After cooling, add 2.5 g of potassium iodide, immediately stopper tightly and shake well. Allow to stand for 5 minutes in a dark place and titrate with 0.1 mol/L sodi-

um thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 1.8217 mg of $C_6H_{14}O_6$

Containers and Storage ***Containers***—Tight containers.

Spectinomycin Hydrochloride Hydrate



$C_{14}H_{24}N_2O_7 \cdot 2HCl \cdot 5H_2O$: 495.35

(1*R*,3*S*,5*R*,8*R*,10*R*,11*S*,12*S*,13*R*,14*S*)-8,12,14-trihydroxy-5-methyl-11,13-bis(methylamino)-2,4,9-trioxatricyclo[8.4.0.03,8]tetradecan-7-one hydrochloride pentahydrate [*22189-32-8*]

Spectinomycin Hydrochloride Hydrate is the hydrochloride of a substance having antibacterial activity produced by the growth of *Streptomyces spectabilis*.
Spectinomycin Hydrochloride Hydrate contains not less than 603 μg (potency) and not more than 713 μg (potency) per mg of spectinomycin ($C_{14}H_{24}N_2O_7$: 332.35).

Description Spectinomycin Hydrochloride Hydrate appears as white to pale yellowish white crystalline powder.
Spectinomycin Hydrochloride Hydrate is freely soluble in water, and practically insoluble in ethanol (95).

Identification (1) To 5 mL of a solution of Spectinomycin Hydrochloride Hydrate (1 in 100) add slowly anthrone TS: a blue to blue-green color is produced at the zone of contact.

(2) Determine the infrared spectra of Spectinomycin Hydrochloride Hydrate and Spectinomycin Hydrochloride RS as directed in the paste method under Infrared Spectrophotometry, both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 3 mL of a solution of Spectinomycin Hydrochloride Hydrate (1 in 150) add 1 drop of silver nitrate TS: a white turbidity is produced.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{20}$: +15 ~ +21° (2.1 g calculated on the anhydrous basis, water, 25 mL, 200 mm)

pH The pH of a solution obtained by dissolving 0.1 g of Streptomycin Hydrochloride Hydrate in 10 mL of water is between 4.0 and 5.6.

Water 16.0 ~ 20.0 % (0.3 g, volumetric titration, direct titration)

Residue on Ignition Not more than 1.0 % (1 g)

Sterility Test It meets the requirement, when Spectinomycin Hydrochloride Hydrate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.09 EU/mg (potency) of spectinomycin, when Spectinomycin Hydrochloride Hydrate is used in a sterile preparation.

Histamine It meets the requirement, when Spectinomycin Hydrochloride Hydrate is used in a sterile preparation. Weigh appropriate an amount of Spectinomycin Hydrochloride Hydrate, dissolve in Isotonic Sodium Chloride Injection, make the solution so that each mL contains 15 mg (potency), and use the solution as the test solution.

Assay Weigh accurately about 30 mg (potency) each of Spectinomycin Hydrochloride Hydrate and Spectinomycin Hydrochloride RS, dissolve each in the 1.0 mL of internal standard solution and 1.0 mL of hexamethyldisilazane with shaking for 1 hour, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 0.5 μL each of these solutions as directed under Gas Chromatography according to the following operating conditions, and determine the ratios, Q_T and Q_S, of the peak area of spectinomycin to that of the internal standard.

Amount [μg (potency)] of spectinomycin ($C_{14}H_{24}N_2O_7$)
= Amount [μg (potency)] of
Spectinomycin Hydrochloride RS $\times \dfrac{Q_T}{Q_S}$

Internal standard solution—Weigh 50 mg of triphenylantimony, dissolve in dimethylformamide to make 25 mL.

Operating conditions

Detector: A hydrogen flame-ionization detector

Column: A glass column, about 3 mm in internal diameter and about 60 cm in length, packed with column gas-chrom Q (80/100 mesh) for gas chromatography impregnated with 5 % SE-52.

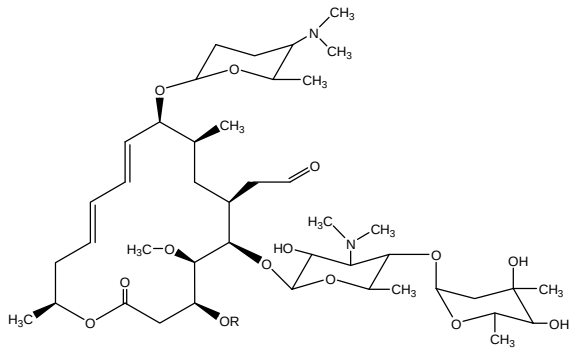
Column temperature: A constant temperature of about 90 °C

Carrier gas: Helium

Flow rate: 45 mL/minute

Containers and Storage ***Containers***—Tight containers.

Spiramycin



Spiramycin I : R = H $C_{43}H_{74}N_2O_{14}$: 843.06
Spiramycin II : R = $COCH_3$ $C_{45}H_{76}N_2O_{15}$: 885.09
Spiramycin III : R = $COCH_2CH_3$ $C_{46}H_{78}N_2O_{15}$: 899.12

Spiramycin I : (4*R*,5*S*,6*R*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-10-{[(2*R*,5*S*,6*R*)-5-(Dimethylamino)-6-methyltetrahydro-2*H*-pyran-2-yl]oxy}-4-hydroxy-9,16-dimethyl-5-methoxy-2-oxo-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-6-yl 3,6-dideoxy-4-*O*-(2,6-dideoxy-3-*C*-methyl-α-L-ribo-hexopyranosyl)-3-(dimethylamino)-α-D-glucopyranoside
Spiramycin II : (4*R*,5*S*,6*R*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-4-Acetyloxy-10-{[(2*R*,5*S*,6*R*)-5-(dimethylamino)-6-methyltetrahydro-2*H*-pyran-2-yl]oxy}-9,16-dimethyl-5-methoxy-2-oxo-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-6-yl 3,6-dideoxy-4-*O*-(2,6-dideoxy-3-*C*-methyl-α-L-ribo-hexopyranosyl)-3-(dimethylamino)-α-D-glucopyranoside
Spiramycin III : (4*R*,5*S*,6*R*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-10-{[(2*R*,5*S*,6*R*)-5-(dimethylamino)-6-methyltetrahydro-2*H*-pyran-2-yl]oxy}-9,16-dimethyl-5-methoxy-2-oxo-4-propanoyloxy-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-6-yl 3,6-dideoxy-4-*O*-(2,6-dideoxy-3-*C*-methyl-α-L-ribo-hexopyranosyl)-3-(dimethylamino)-α-D-glucopyranoside

Spiramycin is a mixture of spiramycin I, spiramycin II, and spiramycin III.
Spiramycin contains not less than 3200 units (potency) per mg of spiramycin I ($C_{43}H_{74}N_2O_{14}$: 843.06), calculated on the dried basis.

Description Spiramycin appears as white to pale yellowish white powder, and has a bitter taste.
Spiramycin is very soluble in methanol, in ethanol (95), and in acetone, sparingly soluble in ether, and very slightly soluble in water.

Identification (1) Dissolve 0.5 g (potency) of Spiramycin in 10 mL of 0.1 mol/L sulfuric acid TS, add 25 mL of water, mix, adjust the pH to 8.0 with 0.1 mol/L sodium hydroxide TS, and add water to make 50 mL. To 5 mL of this solution add 2 mL of a mixture of sulfuric acid and water (2 : 1): a brown color develops.

(2) Dissolve 0.1 g (potency) of Spiramycin in methanol to make 100 mL. To 1 mL of this solution add methanol to make 100 mL, and determine the absorbance between 220 nm and 350 nm of this solution as directed under Ultraviolet-visible Spectrophotomtery: it exhibits maxima at around 232 nm and at around 340 nm.

(3) Proceed with Spiramycin as directed under Purity (2): the principal spot from the test solution shows the same R_f value and color as the principal spot from standard solution S1, and the spots other than the principal spot from the test solution are similar to the spots other than the principal spot from standard solution S1 and different from the principal spot of standard solution S5.

Specific Optical Rotation $[\alpha]_D^{20}$: -80 ~ -85° (1.0 g calculated on the anhydrous basis, 10 % acetic acid solution, 50 mL)

pH Dissolve 0.5 g (potency) of Spiramycin in 5 mL of methanol, and add water to make 100 mL. The pH of this solution is between 8.5 and 10.5.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Spiramycin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Weigh accurately about 40 mg of Spiramycin, dissolve in methanol to make exactly 10 mL, and use this solution as the test solution. Separatey, weigh accurately about 40 mg of Spiramycin RS, and dissolve in methanol to make exactly 10 mL (S1). To 1 mL of this solution add methanol to make exactly 10 mL (S2). Pipet 5 mL and 2 mL of standard solution S2, and dilute with methanol to make 10 mL each (S3, S4). Dissolve 40 mg (potency) of Erythromycin RS in methanol to make exactly 10 mL (S5). Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and standard solutions S1, S2, S3, S4, and S5 on a plate of silica gel for thin-layer chromatography. Develop the plate with the clear supernatant liquid of a mixture of 2-propanol, 15 % ammonium acetate (adjust the pH to 9.6 with 1 mol/L sodium hydroxide TS), and ethyl acetate (4 : 8 : 9) to a distance of about 15 cm, and air-dry the plate. To 10 mL of anisaldehyde add 90 mL of ethanol (95), mix well, add 10 mL of sulfuric acid, and mix well. Spray evenly this solution on the plate, and heat at 110 °C for 5 minutes: standard solution S1 shows the principal spot, and the spots of spiramycin II and spiramycin III appear slightly above and slightly further above the principal spot, respectively. The spot of spiramycin II

from the test solution is not more intense than the spot from standard solution S2, and the spot of spiramycin III from the test solution is not more intense than the spot from standard solution S3. The spots other than the principal spot from the test solution are not more intense than the spot from standard solution S4.

Loss on Drying Not more than 3.5 % (0.5 g, in vacuum, P_5O_2, 80 °C, 6 hours)

Residue on Ignition Not more than 0.1 % (1 g)

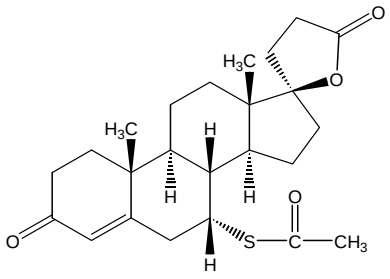
Assay ***The Cylinder-plate method*** (1) Agar media for seed and base layer- Use the culture medium in I 2 1) (3) ① under Microbial Assay for Antibiotics. Adjust the pH of the medium so that it will be between 7.8 and 8.0.

(2) Test organism- *Bacillus subtilis* ATCC 6633

(3) Weigh accurately about 75000 units (potency) of Spiramycin, dissolve in 5 mL of methanol, add 0.1 mol/L phosphate buffer solution (pH 8.0) so that each mL contains 120 units (potency) and 30 units (potency), and use these solutions as the high concentration test solution and low concentration test solution, respectively. Separately, weigh accurately about 75000 units (potency) of Spiramycin RS, dissolve in 5 mL of methanol, add 0.1 mol/L phosphate buffer solution (pH 8.0) so that each mL contains 7500 units (potency), and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 5 °C, and use within 7 days. Pipet a suitable amount of the standard stock solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) so that each mL contains 120 units (potency) and 30 units (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively. Perform the test with these solutions as directed in I 8 under Microbial Assay for Antibiotics.

Containers and Storage ***Containers*****—**Tight containers.

Spironolactone



$C_{24}H_{32}O_4S$: 416.57

7α-Acetylthio-3-oxo-17α-pregn-4-ene-21,17-carbolactone [*52-01-7*]

Spironolactone, when dried, contains not less than 97.0 % and not more than 103.0 % of spironolactone ($C_{24}H_{32}O_4S$).

Description Spironolactone is a white to pale yellow-brown, fine powder.

Spironolactone is freely soluble in chloroform, soluble in ethanol (95), slightly soluble in methanol and practically insoluble in water.

Melting point—198 ~ 207 °C. In a bath at about 125 °C, continue the heating so that the temperature rises at a rate of about 10 °C per minute in the range between 140 °C and 185 °C and when the temperature is near the expected melting range, reduce the heating so that the temperature rises at a rate of about 3 °C per minute.

Identification (1) Determine the absorption spectra of solutions of Spironolactone and Spironolactone RS, in methanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibits exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Spironolactone and Spironolactone RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears between the spectra, dissolve Spironolactone and Spironolactone RS in methanol, respectively, then evaporate methanol to dryness and repeat the test on the residues.

Specific Optical Rotation $[\alpha]_D^{20}$: -33 ~ -37° (after drying, 0.25 g, chloroform, 25 mL, 200 mm).

Purity (1) ***Mercapto compounds***—Shake 2.0 g of Spironolactone with 20 mL of water and filter. To 10 mL of the filtrate, add 1 mL of starch TS and 0.05 mL of 0.01 mol/L iodine VS and mix: a blue color is observed.

(2) ***Related substances***—Dissolve 0.20 g of Spironolactone in 10 mL of ethanol (95) and use this solution as the test solution. Pipet 1 mL of this solution, add ethanol (95) to make exactly 100 mL and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with n-butyl acetate to a distance of about 15 cm and air-dry the plate. Spray evenly a solution of sulfuric acid in methanol (1 in 10) on the plate and heat the plate at 105 °C for 10 minutes: any spot other than the principal spot from the test solution is not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 50 mg each of Spironolactone and Spironolactone RS, previously dried at 105 °C for 2 hours and dissolve separately in methanol to make exactly 250 mL. Pipet 5 mL each of these solutions, add methanol to make exact1y 100 mL and use theses solutions as the test solution and the standard solution, respectively. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry and determine the absorbances, A_T and A_S, of these solutions at 238 nm.

$$\text{Amount (mg) of spironolactone } (C_{24}H_{32}O_4S) = \text{Amount (mg) of Spironolactone RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers*—**Tight containers.

***Storage*—**Light-resistant.

Stannous Fluoride

SnF_2: 156.71

Difluorotin [*7783-47-3*]

Stannous Fluoride contains not less than 71.2 % of stannous tin (Sn^{2+}: 118.71), and not less than 22.3 % and not more than 25.5 % of fluoride (F: 19.00), calculated on the dried basis.

Description Stannous Fluoride is a white crystalline powder, has a bitter and salty taste.
Stannous Fluoride is freely soluble in water and practically insoluble in ethanol (95), ether or chloroform.

Melting point—About 213 °C.

Identification (1) To 5 mL of a solution of Stannous Fluoride in water (1 in 100) in a test tube, add 2 mL of calcium chloride TS: a fine, white precipitate of calcium fluoride is produced.

(2) Mix on a spot plate, 2 drops of a solution of Stannous Fluoride (1 in 100) with 2 drops of silver nitrate TS: a brown-black precipitate is produced.

(3) Add 1 drop of a solution of Stannous Fluoride (1 in 100) to 2 drops of mercury (II) chloride TS: a white, silky precipitate is formed. On further addition of the solution (1 in 100), a brown-black precipitate is produced.

pH Dissolve about 0.1 g of Stannous Fluoride in 25 mL of fresh boiled water: the pH of this solution is between 2.8 and 3.5.

Purity (1) ***Water-insoluble substances*—**Transfer about 10 g of Stannous Fluoride, accurately weighed, to a plastic beaker, add 200 mL of water and stir with a plastic rod for 3 minutes, or until no more solid dissolves. Filter through a tared filtering crucible and wash thoroughly, first with ammonium fluoride solution (1 in 100), then with water. Dry the residue at 105 °C for 4 hours, cool and weigh: not more than 0.2 %.

(2) ***Antimony*—*Rhodamine B solution*—**Dissolve 20 mg of rhodamine B in 200 mL of 0.5 mol/L hydrochloric acid.

Standard solution—Weigh accurately about 55.0 mg of antimony potassium tartrate, dissolve in water to make exactly 200 mL. Pipet 5 mL of this solution, add 6 mol/L hydrochloric acid to make exactly 500 mL and use this solution as the standard solution.

Test solution— Weigh accurately about 1.0 g of Stannous Fuloride, add 6 mol/L hydrochloric acid to make 50 mL and use this solution as the test solution.

Procedure—Pipet 5 mL each of the test solution and the standard solution into separatory funnels, add 15 mL of hydrochloric acid and 1 g of ceric sulfite and allow to stand for 5 minutes, with occasional shaking. Add 0.5 g of hydroxylamine hydrochloride and shake for 1 minute. Pipet 15 mL of isopropyl ether into the mixture, shake for 30 seconds, add 7 mL of water and mix. Cool in a water-bath at room temperature for 10 minutes, shake for 30 seconds, allow the layers to separate and discard the aqueous layer. Add 20 mL of Rodamin B solution, shake for 30 seconds and discard the aqueous layer again. Decant the ether layer from the top of separatory funnel and centrifuge, if necessary, to obtain a clear solution. Perform the test with the ether layer obtained from the test solution and the standard solution at the wavelength of a maximum absorbance at about 550 nm, as directed under Ultraviolet-visible spectrophotometer, using water as the blank: the absorbance of the test solution does not exceed that of the standard solution (no more than 0.005 %).

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 4 hours).

Assay (1) ***Stannous ion*—**Transfer about 0.25 g of Stannous Fluoride, accurately weighed, to an Erlenmeyer flask and add 300 mL of hot, freshly boiled 3 mol/L hydrochloric acid. While passing a stream of an oxygen-free inert gas over the surface of the liquid, swirl the flask of dissolve the Stannous Fluoride and cool to room temperature, Add 5 mL of potassium iodide TS and titrate in an inert atmosphere with 0.05 mol/L potassium iodide-iodate VS, adding 3 mL of starch TS as the endpoint is approached.

Each mL of 0.05 mol/L potassium iodide-iodate VS
= 5.935 mg of Sn^{2+}

(2) ***Fluoride*—**Dissolve about 0.1 g of Stannous Fluoride, accurately weighed, in 50 mL of water, shake

vigorously for 5 minutes and add water to make exactly 250 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL and use this solution as the test solution. Separately, weigh accurately a certain amount of Stannous Fluoride RS, dissolve in water and use this solution as the standard solution, containing about 10 μg of fluorine per mL. Pipet 1.0, 2.0, 3.0 and 4.0 mL volumes of the standard solution in volumetric flasks, add respectively 10.0 mL of Solution D and water to make 100 mL each and mix. Determine the absorbances of each of these solutions and Solution E at 590 nm as directed under Ultraviolet-visible Spectrophotometry, using Solution C as the blank. Subtract the absorbance of Solution E from those of the fluoride-contained solutions and make a standard curve of content of fluoride (μg) with these absorbances. Separately, pipet 5.0 mL of the test solution, handle as the same method above, obtain the fluoride content (μg) of 5 mL of the test solution from the standard curve.

Solution A—Dissolve 3.16 g of trisodium 4,5-dihydroxy-3-(*p*-sulfophenylazo)-2,7-naphthalen disulfonate in 550 mL of water.

Solution B—Dissolve 0.113 g of oxychlorozirconium in 50 mL of water, add 350 mL of hydrochloric acid to make exactly 500 mL.

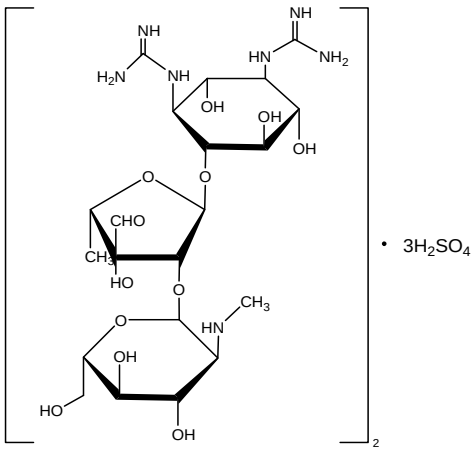
Solution C—Dilute 50 mL of Solution A with 500 mL of water and 35 mL of hydrochloric acid.

Solution D—Mix equal volume of Solution A with Solution B and preserve in brown bottle.

Solution E—To 10.0 mL of Solution D, add water to make exactly 100 mL.

Containers and Storage ***Containers***—Well-closed containers.

Streptomycin Sulfate



$(C_{21}H_{39}N_7O_{12})_2 \cdot 3H_2SO_4$: 1457.38

2-[(1*S*,2*S*,3*R*,4*S*,5*S*,6*R*)-3-(Diaminomethylideneamino)-4-[(2*R*,3*R*,4*R*,5*S*)-3-[(2*S*,3*S*,4*S*,5*R*,6*S*)-4,5-dihydroxy-6-(hydroxymethyl)-3-(methylamino)oxan-2-yl]oxy-4-formyl-4-hydroxy-5-methyloxolan-2-yl]oxy-2,5,6-trihydroxycyclohexyl]guanidine sulfate [*3810-74-0*]

Streptomycin Sulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of *Streptomyces griseus*.
Streptomycin Sulfate contains not less than 740 μg (potency) and not more than 820 μg (potency) of streptomycin ($C_{21}H_{39}N_7O_{12}$: 581.57) per mg, calculated on the dried basis

Description Streptomycin Sulfate appears as white to pale yellowish white powder.
Streptomycin Sulfate is freely soluble in water, and very slightly soluble in ethanol (95).

Identification (1) Dissolve 50 mg of Streptomycin Sulfate in 5 mL of water, add 1 mL of ninhydrin TS and 0.5 mL of pyridine, and heat for 10 minutes: a purple color develops.

(2) Dissolve 10 mg each of Streptomycin Sulfate and Streptomycin Sulfate RS in 10 mL of water, and use these solutions as the test solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of these solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (7 in 100) to a distance of about 12 cm, and air-dry the plate. Spray evenly a mixture of a solution of 1,3-dihydroxynaphthalene in ethanol (95) (1 in 500) and diluted sulfuric acid (1 in 5) (1 : 1) on the plate, and heat at about 150 °C for about 5 minutes: the principal spots obtained from the test solution and standard solution show the same color and R_f value.

(3) A solution of Streptomycin Sulfate (1 in 5) responds to the Qualitative Tests for sulfate.

Specific Optical Rotation $[\alpha]_D^{20}$: -79 ~ -88° (0.5 g calculated on the dried basis, water, 50 mL, 100 mm)

pH The pH of a solution obtained by dissolving 2 g of Streptomycin Sulfate in 10 mL of water is between 4.5 and 7.0.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Streptomycin Sulfate in 5 mL of water: the solution is clear and colorless to pale yellow.

(2) ***Heavy metals***—Proceed with 2.0 g of Streptomycin Sulfate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Proceed with 2.0 g of Streptomycin Sulfate according to Method 3, and perform the test (not more than 1 ppm).

(4) ***Related substances***—Weigh accurately 0.20 g of Streptomycin Sulfate, dissolve in a mixture of methanol and sulfuric acid (97 : 3) to make 5 mL, and heat under a reflux condenser for 1 hour. After cooling, wash the inside of the condenser with a suitable amount of a mixture of methanol and sulfuric acid (97 : 3), add a mixture of methanol and sulfuric acid (97 : 3) to make exactly 20 mL, and use this solution as the test solution. Separately, weigh accurately 36 mg of D(+)-mannose, dissolve in a mixture of methanol and sulfuric acid (97 : 3) to make 5 mL, and heat with a reflux condenser for 1 hour. After cooling, wash the inside of the condenser with a suitable amount of a mixture of methanol and sulfuric acid (97 : 3), add a mixture of methanol and sulfuric acid (97 : 3) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of toluene, methanol, and acetic acid (100) (2 : 1 : 1) to a distance of 13 to 15 cm, and air-dry the plate. Spray evenly a mixture of a solution of 1,3-dihydroxy-naphthalene in ethanol (95) (1 in 500) and diluted sulfuric acid (1 in 5) (1 : 1) on the palte, and heat at 110 °C for 5 minutes: the spot from the test solution corresponding to the spot from the standard solution is not more intense than the spot from the standard solution.

Loss on Drying Not more than 5.0 % (0.5 g, reduced pressure not exceeding 0.67 kPa, 60 °C, 3 hours)

Residue on Ignition Not more than 1.0 % (1 g)

Sterility Test It meets the requirement, when Streptomycin Sulfate is used in a sterile preparation.

Bacterial Endotoxins Less than 0.10 EU/mg of streptomycin, when Streptomycin Sulfate is used in a sterile preparation.

Abnormal Toxicity Dissolve 1 mg of Streptomycin Sulfate in 0.5 mL of water for injection, and inject intravenously for 15 to 30 seconds to each of 5 healthy mice weighing 17 to 24 g. Use animals in which no abnormalities are observed for not less than 5 days prior to the test. No animals die during the 24 hour post-dosage observation. If 1 animal dies, repeat the test with 5 animals: no animals die during the 24 hour observation.

Histamine It meets the requirement, when Streptomycin Sulfate is used in a sterile preparation. Weigh appropriate amount of Streptomycin Sulfate, dissolve in Isotonic Sodium Chloride Injection, make the solution contain 3.0 mg (potency) per mL, and use the solution as the test solution.

Assay ***The Cylinder-plate method*** (1) Agar media for seed and base layer- Use the medium in I 2 1) (1) ① under Microbial Assay for Antibiotics.

(2) Test organism- *Bacillus subtilis* ATCC 6633.

(3) Weigh accurately about 20 mg (potency) of Streptomycin Sulfate, dissolve in water to make exactly 50 mL, and use this solution as the test stock solution. Pipet a suitable amount of the test stock solution, add 0.1 mol/L phosphate buffer solution, pH 8.0, to make solutions so that each mL contains 8.0 μg (potency) and 2.0 μg (potency), and use these solutions as the high concentration test solution and low concentration test solution, respectively. Separately, weigh accurately about 20 mg (potency) of Streptomycin Sulfate RS, previously dried, dissolve in diluted pH 6.0 phosphate buffer solution (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution between 5 °C and 15 °C, and use within 30 days. Pipet a suitable amount of the standard stock solution, add 0.1 mol/L phosphate buffer solution, pH 8.0, to make solutions so that each mL contains 8.0 μg (potency) and 2.0 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively. Perform the test with these solutions as directed in I) (8) under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Streptomycin Sulfate for Injection

Streptomycin Sulfate for Injection is a preparation for injection, which is dissolved before use.
Streptomycin Sulfate for Injection contains not less than 90.0 % and not more than 110.0 % of the labeled amount of streptomycin ($C_{21}H_{39}N_7O_{12}$: 581.57).

Method of Preparation Prepare as directed under Injections, with Streptomycin Sulfate.

Description Streptomycin Sulfate for Injection appears as white or pale yellow masses or powder.

Identification Perform the test as directed in the Identification (2) under Streptomycin Sulfate.

pH The pH of a solution obtained by dissolving an amount of Streptomycin Sulfate for Injection, equivalent to 2.0 g (potency) of streptomycin, in 10 mL of water is between 5.0 and 7.0.

Purity ***Clarity of solution***—Dissolve an amount of Streptomycin Sulfate for Injection, equivalent to 1.0 g (potency) of streptomycin sulfate according to the labeled amount, in 3 mL of water: the solution is clear, and the absorbance of this solution, determined at 400

nm as directed under Ultraviolet-visible Spectrophotometry, is not more than 0.50.

Loss on Drying Not more than 4.0 % (0.5 g, reduced pressure not exceeding 0.67 kPa, 60 °C, 3 hours)

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.10 EU/mg (potency) of streptomycin.

Foreign Insoluble Matter Test It meets the requirement.

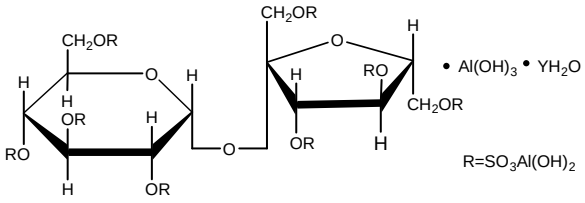
Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Proceed as directed in the Assay under Streptomycin Sulfate. Weigh accurately an amount of Streptomycin Sulfate for Injection, equivalent to about 1.0 g (potency) according to the labeled potency, and add water to make exactly 200 mL. Pipet a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0, so that each mL contains 8.0 μg (potency) and 2.0 μg (potency), and use these solutions as the high concentration test solution and low concentration test solution, respectively.

Containers and Storage ***Containers***—Hermetic containers.

Sucralfate Hydrate



$C_{12}H_{30}Al_8O_{51}S_8 \cdot x\mathrm{Al(OH)_3} \cdot yH_2O$

Aluminum;[(2*R*,3*R*,4*S*,5*R*,6*R*)-2-[(2*R*,3*R*,4*S*,5*S*)-3,4-bis(λ1-alumanyloxysulfonyloxy)-2,5-bis(λ1-alumanyloxysulfonyloxymethyl)oxolan-2-yl]oxy-3,5-bis(λ1-alumanyloxysulfonyloxy)-6-(λ1-alumanyloxysulfonyloxymethyl)oxan-4-yl]oxysulfonyloxyaluminum;tetracontahydrate [*54182-58-0*]

Sucralfate Hydrate contains not less than 17.0 % and not more than 21.0 % of aluminum (Al: 26.98) and not less than 34.0 % and not more than 43.0 % of sucrose octasulfate ester ($C_{12}H_{22}O_{35}S_8$: 982.80), calculated on the dried basis.

Description Sucralfate Hydrate appears as white powder. Sucralfate Hydrate is odorless and tasteless.
Sucralfate Hydrate is practically insoluble in water, hot water, ethanol (95) or ether.
Sucralfate Hydrate dissolves in dilute hydrochloric acid or Sulfuric acid-sodium hydroxide TS.

Identification (1) To 50 mg of Sucralfate Hydrate in a small test tube, add 50 mg of fresh pieces of metallic sodium and melt by careful heating. Immerse the test tube immediately in 100 mL of water, break the test tube, shake well and filter. To 5 mL of the filtrate, add 1 drop of sodium pentacyanonitrosylferrate (III) TS: a red-purple color is observed.

(2) Dissolve 40 mg of Sucralfate Hydrate in 2 mL of dilute sulfuric acid and add gently 2 mL of Anthrone TS to make 2 layers: a blue color develops at the zone of contact and gradually changes to blue-green.

(3) Dissolve 0.5 g of Sucralfate Hydrate in 10 mL of dilute hydrochloric acid: the solution responds to the Qualitative Tests for aluminum.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Sucralfate Hydrate in 10 mL of dilute sulfuric acid: the solution is clear and colorless.

(2) ***Chloride***—Dissolve 0.5 g of Sucralfate Hydrate in 30 mL of dilute nitric acid and heat gently to boiling. After cooling, add water to make 100 mL and to 10 mL of this solution, add 3 mL of dilute nitric acid and water to make 50 mL. Perform the test with this solution as the test solution . Prepare the control solution with 0.70 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.50 %).

(3) ***Heavy metals***—Dissolve 1.0 g of Sucralfate Hydrate in 20 mL of a solution of sodium chloride (1 in 5) and 1 mL of dilute hydrochloric acid and to this solution, add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution as follows: evaporate 1 mL of dilute hydrochloric acid on a water-bath to dryness and add 20 mL of a solution of sodium chloride (1 in 5), 2 mL of dilute acetic acid, 2.0 mL of standard lead solution and water to make 50 mL (not more than 10 ppm).

(4) ***Free aluminum***—To 3.0 g of Sucralfate Hydrate, add 50 mL of water, heat in a water-bath for 5 minutes, cool and filter. Wash the residue with four 5 mL volumes of water, combine the filtrate with the washings, add 2 mL of dilute hydrochloric acid and heat in a water-bath for 30 minutes. After cooling, neutralize the solution with Sodium hydroxide TS, add water to make exactly 100 mL and use this solution as the test solution. Pipet 50 mL of the test solution, add exactly 25 mL of 0.05 mol/L disodium ethylenediaminetetraacetate VS and 20 mL of acetic acid-ammomum acetate buffer solution, pH 4.5 and boil for 5 minutes. After cooling, add 50 mL of ethanol (95) and titrate the excess disodium ethylenediaminetetraacetate with 0.05 mol/L zinc ace-

tate VS until the color of the solution changes from green-purple through purple to red (indicator: 3 mL of Dithizone TS). Perform a blank determination and make any necessary correction (not more than 0.2 %).

Each mL of 0.05 mol/L
disodium ethylene-diaminetetraacetate VS
= 1.3491 mg of Al

(5) ***Arsenic***—Dissolve 1.0 g of Sucralfate Hydrate in 5 mL of dilute hydrochloric acid, use this solution as the test solution and perform the test (not more than 2 ppm).

(6) ***Related substances***—Proceed with 50 μL of the test solution obtained in the Assay (2) under Sucrose octasulfate ester as directed in the Assay (2) under Sucrose octasulfate ester and perform the test as directed under Liquid Chromatography. Determine the peak area of sucrose octasulfate ester from the test solution and that of a related substance with the relative retention time about 0.7 to the peak of sucrose octasulfate ester by the automatic integration method and calculate the ratio of the peak area of the related substance to that of sucrose octasulfate ester: it is not more than 0.1.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of sucrose octasulfate ester from 50 μL of the standard solution obtained in the Assay (2): Sucrose octasulfate ester composes 60 % to 100 % of the full scale.

Loss on Drying Not more than 14.0 % (1 g, 105 °C, 3 hours).

Acid-Consuming Capacity Weigh accurately about 0.25 g of Sucralfate Hydrate, previously dried and perform the test: the amount of 0.1 mol/L hydrochloric acid VS consumed per g of Sucralfate Hydrate is not less than 130 mL.

Assay (1) ***Aluminum***—Weigh accurately about 1 g of Sucralfate Hydrate, dissolve in 10 mL of dilute hydrochloric acid by warming on a water-bath, cool and add water to make exactly 250 mL. Pipet 25 mL of this solution, add exactly 25 mL of 0.05 mol/L disodium ethylenediaminetetraacetate VS and 20 mL of acetic acid-ammonium acetate buffer solution, pH 4.5 and boil for 5 minutes. After cooling, add 50 mL of ethanol (95) and titrate the excess disodium ethylenediaminetetraacetate with 0.05 mol/L zinc acetate VS until the color of the solution changes from green-purple through purple to red (indicator: 3 mL of Dithizone TS). Perform a blank determination and make any necessary correction.

Each mL of 0.05 mol/L
disodium ethylenediaminetetraacetate VS
= 1.3491 mg of Al

(2) ***Sucrose octasulfate ester***—Weigh accurately about 0.55 g of Sucralfate Hydrate, add exactly 10 mL of Sulfuric acid-sodium hydroxide TS, shake vigorously and dissolve with ultrasonic wave at below 30 °C for 5 minutes. To this solution, add 0.1 mol/L sodium hydroxide VS to make exactly 25 mL and use this solution as the test solution. Separately, weigh accurately about 0.25 g of Potassium Sucrose Octasulfate RS, add the mobile phase to make exactly 25 mL and use this solution as the standard solution. Prepare rapidly the test solution and the standard solution and perform the test immediately. Proceed with 50 μL each of these solutions as directed under Liquid Chromatography according to the following conditions. Determine the peak areas, A_T and A_S, of sucrose octasulfate ester for the test solution and the standard solution, respectively.

Amount (mg) of sucrose octasulfate ester ($C_{12}H_{22}O_{35}S_8$)
= Amount (mg) of Potassium Sucrose Octasulfate RS,

calculated on the dried basis $\times \frac{A_T}{A_S} \times 0.7633$

Operating conditions

Detector: A differential refractometer.

Column: A stainless steel column, about 4 mm in internal diameter and about 30 cm in length, packed with aminopropylsilanized silica gel for liquid chromatography (about 8 μm in particle diameter).

Mobile phase: Dissolve a suitable amount (26 g to 132 g) of ammonium sulfate in 1000 mL of water and adjust with phosphoric acid to a pH of 3.5. Allow a solution of Potassium Sucrose Octasulfate RS in dilute hydrochloric acid (1 in 100) to stand at 60 °C for 10 minutes, cool and perform the test immediately. Adjust the amount of ammonium sulfate in the mobile phase so that the peak of a related substance with the relative retention time being about 0.7 to that of sucrose octasulfate ester almost becomes close to the base line and the peak of sucrose octasulfate ester elutes most rapidly.

Flow rate: Adjust the flow rate so that the retention time of sucrose octasulfate ester is between 6 and 11 minutes.

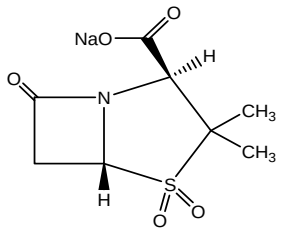
System suitablilty

System performance: Allow a solution of Potassium Sucrose Octasulfate RS in dilute hydrochloric acid (1 in 100) to stand at 60 °C for 10 minutes and cool. When the procedure is run immediately with 50 μL of this solution under the above operating conditions, the resolution between sucrose octasulfate ester and a related substance with the relative retention time being about 0.7 to sucrose octasulfate ester is not less than 1.5.

System repeatibility: When the test is repeated 6 times with 50 μL each of the standard solution under the above operating conditions: the relative standard deviation of the peak area of sucrose octasulfate ester is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Sulbactam Sodium



$C_8H_{10}NNaO_5S$: 255.22

Sodium (3*S*)-2,2-dimethyl-1,1-dioxo-1λ6-penam-3-carboxylate [*69388-84-7*]

Sulbactam Sodium contains not less than 875 μg (potency) and not more than 941 μg (potency) per mg of sulbactam ($C_8H_{11}NO_5S$: 233.24), calculated on the anhydrous basis

Description Sulbactam Sodium is a white to yellowish white crystalline powder.
Sulbactam Sodium is freely soluble in water, sparingly soluble in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in acetonitrile.

Identification (1) Determine the infrared spectra of Sulbactam Sodium and Sulbactam Sodium RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Sulbactam Sodium responds to the Qualitative Tests (1) for sodium salt.

Crystallinity Test It meets the requirement.

Specific Optical Rotation $[\alpha]_D^{20}$: +219 ~ +233°(1.0 g, water, 100 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g of Sulbactam Sodium in 20 mL of water is between 5.2 and 7.2.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Sulbactam Sodium in 20 mL of water: the solution is clear and colorless to pale yellow.

(2) ***Heavy metals***—Proceed with 1.0 g of Sulbactam Sodium according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Proceed with 1.0 g of Sulbactam Sodium according to Method 3, and perform the test (not more than 2 ppm).

(4) ***Sulbactam penicillamine***—Weigh accurately about 0.2 g of Sulbactam Sodium, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the test solution. Separately, weigh accurately about 40 mg of sulbactam sodium for sulbactam penicillamine, dissolve in 2 mL of water, add 0.5 mL of sodium hydroxide TS, allow to stand at room temperature for 10 minutes, add 0.5 mL of 1 mol/L hydrochloric acid TS, and add the mobile phase to make exactly 100 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of sulbactam penicillamine in each solution: the amount of sulbactam penicillamine is not more than 1.0 %.

$$\text{Amount (\%) of sulbactam penicillamine} = \frac{W_S}{W_T} \times \frac{A_T}{A_S} \times 5$$

W_S: Amount (mg) of sulbactam sodium for sulbactam penicillamine taken
W_T: Amount (mg) of Sulbactam Sodium taken

Operating conditions
Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.
Detector: An ultraviolet absorption photometer (wavelength: 230 nm)
System suitability
System performance: Proceed as directed in the system suitability in the Assay.
System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of sulbactam penicillamine is not more than 2.0 %.

Water Not more than 1.0 % (0.5 g, volumetric titration, direct titration)

Sterility Test It meets the requirement, when Sulbactam Sodium is used in a sterile preparation.

Bacterial Endotoxins Less than 0.17 EU/mg (potency) of sulbactam, when Sulbactam Sodium is used in a sterile preparation.

Assay Weigh accurately about 0.1 g of Sulbactam Sodium and Sulbactam Sodium RS, dissolve each in a suitable amount of the mobile phase, add exactly 10.0 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following operating conditions, and determine the

ratios, Q_T and Q_S, of the peak area of sulbactam to that of the internal standard.

$$\text{Amount [μg (potency)] of sulbactam } (C_8H_{11}NO_5S) = \text{Amount [μg (potency)] of Sulbactam RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of ethyl parahydroxybenzoate in the mobile phase (7 in 1000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column, about 3.9 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter)

Column temperature: A constant temperature of about 35 °C

Mobile phase: To 250 mL of acetonitrile add 0.005 mol/L tetrabutylammonium hydroxide TS to make exactly 1000 mL

Flow rate: Adjust the flow rate so that the retention time of sulbactam is about 6 minutes.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, sulbactam and the internal standard are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of sulbactam is not more than 1.0 %.

0.005 mol/L Tetrabutylammonium hydroxide TS—To 10 mL of tetrabutylammonium hydroxide TS add 700 mL of water, adjust the pH to 4.0 with diluted phosphoric acid (1 in 10), and add water to make 1000 mL.

Containers and Storage ***Containers***—Tight containers.

Sulbactam Sodium·Ampicillin Sodium for Injection

Sulbactam Sodium·Ampicillin Sodium for Injection is a preparation for injection, which is dissolved before use.

Sulbactam Sodium·Ampicillin Sodium for Injection contains not less than 90.0 % and not more than 120.0 % of the labeled amount of sulbactam ($C_8H_{11}NO_5S$: 233.24) and ampicillin ($C_{16}H_{19}N_3O_4S$: 349.41).

Method of Preparation Prepare as directed under Injections so that Sulbactam Sodium·Ampicillin Sodium for Injection contains Sulbactam Sodium and Ampicillin Sodium in the ratio of 1 : 2 (potency).

Description Sulbactam Sodium·Ampicillin Sodium for Injection appears as white to grayish white crystalline powder.

Identification (1) ***Sulbactam sodium***—The retention time of the principal peak of sulbactam from the test solution corresponds to that of the principal peak of sulbactam from the standard solution, as obtained in the Assay (1).

(2) ***Ampicillin sodium***—The retention time of the principal peak of ampicillin from the test solution corresponds to that of the principal peak of ampicillin from the standard solution, as obtained in the Assay (2).

pH The pH of a solution prepared by dissolving an amount of Sulbactam Sodium·Ampicillin Sodium for Injection, equivalent to 1.0 g (potency) of ampicillin sodium, in 100 mL of water is between 8.0 and 10.0.

Water Not more than 2.0 % (0.5 g, volumetric titration, direct titration)

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.20 EU/mg (potency) of ampicillin.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Dissolve Sulbactam Sodium·Ampicillin Sodium for Injection in water according to the labeled amount. Pipet a suitable amount of this solution, add the mobile phase so that each mL contains 0.25 mg (potency) of sulbactam and 0.5 mg (potency) of ampicillin, and use this solution as the test solution. Separately, weigh accurately a suitable amount each of Ampicillin RS and Sulbactam RS, dissolve in the mobile phase so that each mL contains 0.25 mg (potency) of sulbactam and 0.5 mg (potency) of ampicillin, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_{T1}, A_{S1}, A_{T2}, and A_{S2}, of sulbactam and ampicillin in the test solution and standard solution.

$$\text{Amount [μg (potency)] of sulbactam } (C_8H_{11}NO_5S)$$

= Concentration of sulbactam in the standard solution [μg (potency)/mL]

$$\times \frac{A_{T1}}{A_{S1}} \times \text{Test solution dilution factor}$$

Amount [μg (potency)] of ampicillin ($C_{16}H_{19}N_3O_4$)
= Concentration of ampicillin in the standard solution [μg (potency)/mL]

$$\times \frac{A_{T2}}{A_{S2}} \times \text{Test solution dilution factor}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column about 4 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (1.5 to 10 μm in particle diameter).

Mobile phase: a mixture of 0.005 mol/L tetrabutylammonium hydroxide TS and acetonitrile (1650 : 350).

Flow rate: 2.0 mL/minute.

0.005 mol/L Tetrabutylammonium hydroxide TS—To 6.6 mL of a 40 % solution of tetrabutylammonium hydroxide add water to make 1800 mL, adjust the pH to 5.0 ± 1 with 1 mol/L phosphoric acid, and add water to make 2000 mL.

System suitability

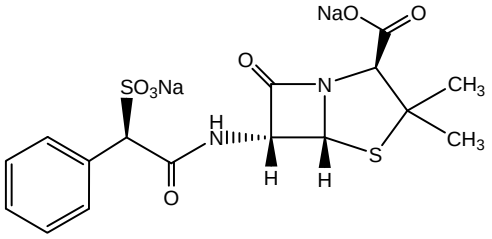
System performance: When the procedure is run with 10 μL of the system suitability solution under the above operating conditions, the relative retention time of ampicillin with respect to sulbactam alkaline degredation product is about 0.7, with the resolution between these peaks being not less than 4.0. When the procedure is run with 10 μL of the standard solution under the above operating conditions, the relative retention time of ampicillin with respect to sulbactam is about 0.35. The number of theoretical plates and symmetry factor of sulbactam are not less than 3500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 5 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of sulbactam is not more than 2.0 %.

System suitability solution—Dissolve about 30 mg of Sulbactam RS in 0.01 mol/L sodium hydroxide solution to make 100 mL, allow to stand for 30 minutes, and adjust the pH to 5.0 ± 1 with 1 mol/L phosphoric acid. To 5 mL of this solution add 4.25 mL of acetonitrile and 0.005 mol/L tetrabutylammonium hydroxide TS to make 25 mL. To 1 mL of this solution add 15 mg of Ampicillin RS and add the mobile phase to make 25 mL.

Containers and Storage ***Containers***—Hermetic containers.

Sulbenicillin Sodium



$C_{16}H_{16}N_2Na_2O_7S_2$: 458.42

Disodium (3*S*)-2,2-dimethyl-6b-[(2*R*)-2-phenyl-2-sulfonatoacetamido]penam-3-carboxylate [*28002-18-8*]

Sulbenicillin Sodium contains not less than 900 μg (potency) and not more than 970 μg (potency) per mg of sulbenicillin ($C_{16}H_{18}N_2O_7S_2$: 414.45), calculated on the anhydrous basis.

Description Sulbenicillin Sodium appears as white to pale yellowish white powder.
Sulbenicillin Sodium is very soluble in water, freely soluble in methanol, and slightly soluble in ethanol (99.5).
Sulbenicillin Sodium is hygroscopic.

Identification (1) Determine the infrared spectra of Sulbenicillin Sodium and Sulbenicillin Sodium RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Sulbenicillin Sodium responds to the Qualitative Tests (1) for sodium salt.

Specific Optical Rotation $[\alpha]_D^{20}$: +167 ~ +182° (1 g calculated on the anhydrous basis, water, 20 mL, 100 mm)

pH The pH of a solution obtained by dissolving 0.20 g of Sulbenicillin Sodium in 10 mL of water is between 4.5 and 7.0.

Purity (1) ***Clarity and color of solution***—Dissolve 2.5 g of Sulbenicillin Sodium in 5 mL of water: the solution is clear and colorless to pale yellow.

(2) ***Heavy metals***—Proceed with 1.0 g of Sulbenicillin Sodium according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Proceed with 1.0 g of Sulbenicillin Sodium according to Method 1, and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 0.1 g of Sulbenicillin Sodium in 15 mL of the mobile phase,

and use this solution as the test solution. Perform the test with 10 μL of the test solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of these peak by the area percentage method: the area of the each peak other than the two peaks of sulbenicllin is not more than 2.0 %, and the total area of the peaks other than the two peaks of sulbenicillin is not more than 5.0 %.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 3.9 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Dissolve 10 g of potassium dihydrogen phosphate in 750 mL of water, adjust the pH to 6.0 ± 0.1 with sodium hydroxide TS, and add water to make 1000 mL. To 940 mL of this solution add 60 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of the lately eluted peak among the two peaks of sulbenicillin is 18 minutes.

System suitability

Test for required detectability: Pipet 1 mL of the test solution, add the mobile phase to make exactly 100 mL, and use this solution as the system suitability solution. Pipet 1 mL of the system suitability solution, and add the mobile phase to make exactly 10 mL. Confirm that the total area of the two peaks of sulbenicillin obtained from 10 μL of this solution is equivalent to 7 to 13 % of that from the system suitability solution.

System performance: When the procedure is run with 10 μL of the test solution under the above operating conditions, the resolution between the two peaks of sulbenicillin is not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the total areas of the two peaks of sulbenicillin is not more than 5.0 %.

Time span of measurement: About 1.5 times as long as the retention time of the lately eluted peak among the two peaks of sulbenicillin beginning after the solvent peak

Water Not more than 6.0 % (0.5 g, volumetric titration, direct titration)

Sterility Test It meets the requirement, when Sulbenicillin Sodium is used in a sterile preparation.

Bacterial Endotoxins Less than 0.05 EU/mg (potency) of sulbenicillin, when Sulbenicillin Sodium is used in a sterile preparation.

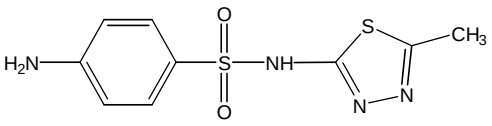
Assay ***The Cyliner-plate method*** (1) Agar medium for seed and base layer- Use the culture medium in I 2 1) (1) ① under Microbial Assay for Antibiotics. Adjust the pH of the medium so that it will be between 6.4 and 6.6 after sterilization.

(2) Test organism- *Bacillus subtilis* ATCC 6633

(3) Weigh accurately an amount of Sulbenicillin Sodium, equivalent to about 50 mg (potency), and dissolve in phosphate buffer solution (pH 6.0) to make exactly 50 mL. Pipet a suitable amount of this solution, add phosphate buffer solution (pH 6.0) so that each mL contains 40 μg (potency) and 10 μg (potency), and use these solutions as the high concentration test solution and low concentration test solution, respectively. Separately, weigh accurately an amount of Sulbenicillin Sodium RS, equivalent to about 50 mg (potency), dissolve in phosphate buffer solution (pH 6.0) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution in a freezer, and use within 4 days. Pipet a suitable amount of the standard stock solution, add phosphate buffer solution (pH 6.0) so that each mL contains 40 μg (potency) and 10 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively. Perform the test with these solutions as directed in I 8 under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Hermietic containers.

Sulfamethizole



$C_9H_{10}N_4O_2S_2$: 270.33

4-Amino-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)benzenesulfonamide [*144-82-1*]

Sulfamethizole, when dried, contains not less than 99.0 % and not more than 101.0 % of sulfamethizole ($C_9H_{10}N_4O_2S_2$).

Description Sulfamethizole appears as white to yellowish white crystals or crystalline powder and is odorless.

Sulfamethizole is slightly soluble in acetic acid (100) or in ethanol (95) and practically insoluble in water or in ether.

Sulfamethizole dissolves in dilute hydrochloric acid or sodium hydroxide TS.

Sulfamethizole is gradually colored by light.

Identification Determine the infrared spectra of

Sulfamethizole and Sulfamethizole RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 208 ~ 211 °C.

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Sulfamethizole in 3 mL of sodium hydroxide TS and 20 mL of water: the solution is clear and colorless.

(2) ***Acid***—To 1.0 g of Sulfamethizole, add 50 mL of water, warm at 70 °C for 5 minutes, allow to stand for 1 hour in an ice-bath and filter. To 25 mL of the filtrate, add 2 drops of methyl red TS and 0.60 mL of 0.1 mol/L sodium hydroxide VS: a yellow color is observed.

(3) ***Heavy metals***—Proceed with 1.0 g of Sulfamethizole according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Arsenic***—Prepare the test solution with 1.0 g of Sulfamethizole according to Method 3 and perform the test (not more than 2 ppm).

(5) ***Related substances***—Dissolve 0.10 g of Sulfamethizole in acetone to make 10 mL and use this solution as the test solution. Pipet 1.0 mL of the test solution, add acetone to make exactly 50 mL, then pipet 5.0 mL of this solution, add acetone to make exactly 20 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and glacial acetic acid (20 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than that from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

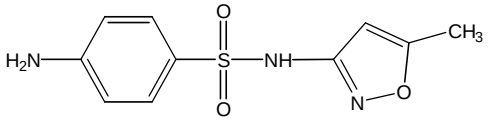
Assay Weigh accurately about 0.4 g of Sulfamethizole, previously dried, dissolve in 5 mL of hydrochloric acid and 50 mL of water, add 10 mL of a solution of potassium bromide (3 in 10), cool below 15 °C and titrate with 0.1 mol/L sodium nitrite VS (potentiometric titration or amperometric titration, Endpoint Detection Methods in Titrimetry).

Each mL of 0.1 mol/L sodium nitrite VS
= 27.033 mg of $C_9H_{10}N_4O_2S_2$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Sulfamethoxazole



Sulfisomezole $C_{20}H_{11}N_3O_3S$: 253.28

4-Amino-*N*-(5-methyl-1,2-oxazol-3-yl)benzenesulfonamide [*723-46-6*]

Sulfamethoxazole, when dried, contains not less than 99.0 % and not more than 101.0 % of sulfamethoxazole ($C_{20}H_{11}N_3O_3S$).

Description Sulfamethoxazole appears as white crystals or ctystalline powder, is odorless and has a slightly bitter taste.
Sulfamethoxazole is very soluble in *N,N*-dimethylformamide, sparingly soluble in ethanol (95), slightly soluble in ether and very slightly soluble in water.
Sulfamethoxazole dissolves in sodium hydroxide TS.
Sulfamethoxazole is gradually colored by light.

Identification Determine the infrared spectra of Sulfamethoxazole and Sulfamethoxazole RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 169 ~ 172 °C.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Sulfamethoxazole in 5 mL of sodium hydroxide TS and 20 mL of water: the solution is clear and colorless.

(2) ***Acids***—To 1.0 g of Sulfamethoxazole, add 50 mL of water, warm at 70 °C for 5 minutes, allow to stand in an ice-bath for 1 hour and filter. To 25 mL of the filtrate, add 2 drops of methyl red TS and 0.60 mL of 0.1 mol/L sodium hydroxide TS: a yellow color is observed.

(3) ***Heavy metals***—Proceed with 1.0 g of Sulfamethoxazole according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Arsenic***—Prepare the test solution with 1.0 g of Sulfamethoxazole according to Method 3 and perform the test (not more than 2 ppm).

(5) ***Selenium***—Proceed with 0.2 g of Sulfamethoxazole as directed under Oxygen Flask Combustion Method, using 25 mL of diluted nitric acid (1 in 30) as the absorbing liquid. Use a 1000 mL com-

bustion flask. After combustion, wash the stopper and the inside of the flask with 10 mL of water. Transfer the liquid inside the flask to a 150 mL beaker, using about 20 mL of water. Heat gently to boil, boil for 10 more minutes, cool to room temperature, and use this solution as the test solution. Separately, pipet 6.0 mL of selenium standard stock solution, add 25 mL of diluted nitric acid (1 in 30) and 25 mL of water, and use this solution as the standard solution. Adjust the pH of the test solution and standard solution to 2.0 with diluted ammonia solution (28) (1 in 2), dilute with water to make 60 mL, transfer to a separatory funnel using 10 mL of water, and wash the separatory funnel with 10 mL of water. Add 0.2 g of hydroxylamine hydrochloride, stir to dissolve, add immediately 5.0 mL of 2,3-diamino-naphthalene TS, stopper, mix by stirring, and allow to stand at room temperature for 100 minutes. Add 5.0 mL of cyclohexane, shake vigorously for 2 minutes, allow the layers to separate, discard the water layer, centrifuge the cyclohexane extract to remove water, and take the cyclohexane layer. Determine the absorbances at 380 nm of these solutions as directed under Ultraviolet-visible Spectrophotometry, using a solution prepared by adding 25 mL of water to 25 mL of diluted nitric acid (1 in 30) and proceeding in the same manner, as the blank: the absorbance obtained from the test solution is not more than that from the standard solution (not more than 30 ppm).

(6) ***Related substances***—Dissolve 0.2 g of Sulfamethoxazole in ammonia solution (28) methanol solution (1 in 50) to make 10 mL and use this solution as the test solution. Pipet 1.0 mL of the test solution, add ammonia solution (28) methanol solution (1 in 50) to make exactly 10 mL. Pipet 1.0 mL of this solution, add ammonia solution (28) methanol solution (1 in 50) to make exactly 20 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-later Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetonitrile and diluted ammonia water (7 in 100) (10 : 8 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

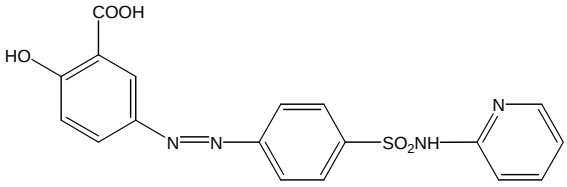
Assay Weigh accurately 0.4 g of Sulfamethoxazole, previously dried, dissolve in 30 mL of *N,N*-dimethylformamide and 10 mL of water. Titrate with 0.1 mol/L of sodium hydroxide VS (indicator: 0.5 mL of thymolphthalein TS) until the color of the solution is pale blue. Separately, perform a blank determination using a mixture of 30 mL *N,N*-dimethylformamide and 26 mL of water and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 25.328 mg $C_{20}H_{11}N_3O_3S$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Sulfasalazine



Salazosulfapyridine $C_{18}H_{14}N_4O_5S$: 398.39

6-Oxo-3-[[4-(pyridin-2-ylsulfamoyl)phenyl] hydrazinylidene]cyclohexa-1,4-diene-1-carboxylic acid [*599-79-1*]

Sulfasalazine, when dried, contains not less than 96.0 % and not more than 101.0 % of sulfasalazine ($C_{18}H_{14}N_4O_5S$).

Description Sulfasalazine is a yellow to yellow-brown, fine powder, is odorless and tasteless.
Sulfasalazine is sparingly soluble in pyridine, slightly soluble in ethanol (95), practically insoluble in water, in chloroform or in ether.
Sulfasalazine dissolves in sodium hydroxide TS.

Melting point—240 ~ 249 °C (with decomposition).

Identification (1) Dissolve 0.1 g of Sulfasalazine in 20 mL of dilute sodium hydroxide TS: a red-brown color is observed. This color gradually fades upon gradual addition of 0.5 g of sodium hydrosulfite with shaking. Use this solution in the following Identification (2) to Identification (4).

(2) To 1 mL of the solution obtained in Identification (1), add 40 mL of water, neutralize with 0.1 mol/L hydrochloric acid TS and add water to make 50 mL. To 5 mL of this solution, add 2 to 3 drops of dilute iron (III) chloride TS: a red color develops and changes to purple, then fades when dilute hydrochloric acid is added dropwise.

(3) The solution obtained in Identification (1) responds to the Qualitative Tests for primary aromatic amines.

(4) To 1 mL of the solution obtained in Identification (1), add 1 mL of pyridine and 2 drops of copper (II) sulfate TS and shake. Add 3 mL of water and 5 mL of chloroform, shake and allow to stand: a green color is observed in the chloroform layer.

(5) Determine the absorption spectra of the solu-

tions of Sulfasalazine and Sulfasalazine RS in dilute sodium hydroxide TS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) ***Chloride***—Dissolve 2.0 g of Sulfasalazine in 12 mL of sodium hydroxide TS and 36 mL of water, add 2 mL of nitric acid, shake and filter. To 25 mL of the filtrate, add 6 mL of dilute nitric acid and water to make 50 mL and perform the test. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014 %).

(2) ***Sulfate***—Dissolve 2.0 g of Sulfasalazine in 12 mL of sodium hydroxide TS and 36 mL of water, add 2 mL of hydrochloric acid, shake and filter. To 25 mL of the filtrate, add 1 mL of dilute hydrochloric acid and water to make 50 mL and Perform the test. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048 %).

(3) ***Heavy metals***—Proceed with 1.0 g of Sulfasalazine according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Arsenic***—Take 1.0 g of Sulfasalazine in a decomposition flask, add 20 mL of nitric acid and heat gently until becomes fluid. After cooling, add 5 mL of sulfuric acid and heat until white fumes are evolved. Add, if necessary, 5 mL of nitric acid after cooling and heat again. Repeat this operation until the solution becomes colorless to pale yellow. After cooling, add 15 mL of a saturated solution of ammonium oxalate and heat until white fumes are evolved again. After cooling, add water to make 25 mL. Perform the test with 5 mL of this solution as the test solution: the color of the test solution is not deeper than that of the following standard solution (not more than 10 ppm).

Standard solution—Proceed in the same manner without Sulfasalazine, transfer 5 mL of the obtained solution to a generator bottle, add exactly 2 mL of standard arsenic solution and proceed in the same manner as the test solution.

(5) ***Related substances***—Dissolve 0.20 g of Sulfasalazine in 20 mL of pyridine and use this solution as the test solution. Pipet 1.0 mL of this solution, add pyridine to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with diluted methanol (9 in 10) to a distance of about 10 cm and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spots from the standard solution.

(6) ***Salicylic acid***—To 0.10 g of Sulfasalazine, add 15 mL of ether and shake vigorously. Add 5 mL of dilute hydrochloric acid, shake vigorously for 3 minutes, collect the ether layer and filter. To the water layer, add 15 mL of ether, shake vigorously for 3 minutes, collect the ether layer, filter and combine the filtrates. Wash the residue on the filter paper with a small quantity of ether and combine the washings and the filtrate. Evaporate the ether with the aid of air-stream at room temperature. To the residue, add dilute ammonium iron (III) sulfate TS, shake and filter, if necessary. Wash the residue on the filter paper with a small quantity of dilute ammonium iron (III) sulfate TS, combine the washings and the filtrate, add dilute ammonium iron (III) sulfate TS to make exactly 20 mL and use this solution as the test solution. Separately, weigh accurately 10 mg of Salicylic Acid RS, previously dried in a desiccator (silica gel) for 3 hours, dissolve in dilute ammonium iron (III) sulfate TS to make exactly 400 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 535 nm as directed under Ultraviolet-visible Spectrophotometry, using dilute ammonium iron (III) sulfate TS as the blank: salicylic acid content is not more than 0.5 %.

$$\text{Content (\%) of salicylic acid } (C_7H_6O_3) = \text{Amount (mg) of Salicylic Acid RS} \times \frac{A_T}{A_S} \times 0.05$$

Loss on Drying Not more than 2.0 % (1 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.2 % (1 g).

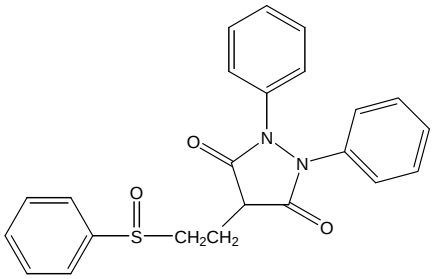
Assay Weigh accurately 20 mg of Sulfasalazine, previously dried and perform the test as directed in the procedure of determination for sulfur under the Oxygen Flask Combustion Method, using 10 mL of diluted strong hydrogen peroxide water (1 in 40) as an absorbing liquid.

$$\text{Each mL of 0.005 mol/L barium perchlorate VS} = 1.9920 \text{ mg of } C_{18}H_{14}N_4O_5S$$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Sulfinpyrazone



$C_{23}H_{20}N_2O_3S$: 404.48

4-[2-(Benzenesulfinyl)ethyl]-1,2-diphenylpyrazolidine-3,5-dione [*57-96-5*]

Sulfinpyrazone, when dried, contains not less than 98.5 % and not more than 101.0 % of sulfinpyrazone ($C_{23}H_{20}N_2O_3S$).

Description Sulfinpyrazone is a white to pale yellowish white powder, is odorless and has a bitter taste.
Sulfinpyrazone is freely soluble in acetic acid (100) or in acetone, soluble in ethanol (95), slightly soluble in ether and practically insoluble in water.
Sulfinpyrazone dissolves in sodium hydroxide TS.

Melting point—About 138 °C (with decomposition).

Identification (1) Dissolve 2 mg of Sulfinpyrazone in 1 mL of acetic acid (100), add 1 mL of palladium (II) chloride TS and 2 mL of chloroform and shake: a yellow color is observed in the chloroform layer.

(2) Determine the absorption spectra of the solutions of Sulfinpyrazone and Sulfinpyrazone RS in 0.01 mol/L sodium hydroxide TS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Sulfinpyrazone and Sulfinpyrazone RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of Sulfinpyrazone in 10 mL of acetone: the solution is clear and colorless. Dissolve 0.5 g of Sulfinpyrazone in 10 mL of sodium hydroxide TS: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 2.0 g of Sulfipyrazone according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Sulfinpyrazone according to Method 3 and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissovle 0.10 g of Sulfinpyrazone in 5 mL of acetone and use this solution as the test solution. Pipet 1.0 mL of the test solution, add acetone to make exactly 100 mL and use this solution as the standard solution (1). Pipet 1.0 mL of the test solution, add acetone to make exactly 200 mL and use this solution as the standard solution (2). Perform the test with the test solution and the standard solutions (1) and (2) as directed under the Thin-layer Chromatography. Spot rapidly 5 μL each of the test solution and the standard solutions (1) and (2) on a plate of silica gel with a fluorescent indicator for thin-layer chromatography under a stream of nitrogen. Develop the plate with a mixture of chloroform and acetic acid (100) (4 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the most intense spot other than the principal spot from the test solution is not more intense than the spot from the standard solution (1) and the spots other than the principal and above spots from the test solution are not more intense than the spot from the standard solution (2).

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 2 hours).

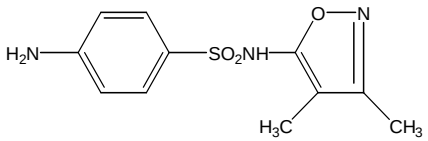
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.5 g of Sulfinpyrazone, previously dried, dissolve in 40 mL of acetone, add 40 mL of water and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration, Endpoint Detection Methods in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 40.45 mg of $C_{23}H_{20}N_2O_3S$

Containers and Storage ***Containers***—Well-closed containers.

Sulfisoxazole



Sulfafurazole $C_{11}H_{13}N_3O_3S$: 267.30

4-Amino-*N*-(3,4-dimethylisoxazol-5-yl)benzenesulfonamide [*127-69-5*]

Sulfisoxazole, when dried, contains not less than 99.0 % and not more than 101.0 % of sulfisoxazole ($C_{11}H_{13}N_3O_3S$).

Description Sulfisoxazole appears as white crystals

or crystalline powder, is odorless and has a slightly bitter taste.
Sulfisoxazole is freely soluble in pyridine or in n-butylamine, soluble in methanol, sparingly soluble in ethanol (95), slightly soluble in acetic acid (100) and very slightly soluble in water or in ether.
Sulifisoxazole dissolves in dilute hydrochloric acid, sodium hydroxide TS or ammonia TS.
Sulifisoxazole is gradually colored by light.

Identification (1) Dissolve 0.01 g of Sulfisoxazole in 1 mL of dilute hydrochloric acid and 4 mL of water: the solution responds to the Qualitative Tests for primary aromatic amines.

(2) Dissolve 0.02 g of Sulfisoxazole in 5 mL of water and 1 mL of *n*-butylamine, add 2 to 3 drops of copper sulfate TS, and shake well. Add 5 mL of chloroform, shake, and allow to stand: a blue-green color develops in the chloroform layer.

(3) Dissolve 0.01 g of Sulfisoxazole in 1 mL of pyridine, add 2 drops of copper sulfate TS, and shake well. Add 3 mL of water and 5 mL of chloroform, shake, and allow to stand: a pale yellow-brown color develops in the chloroform layer.

(4) To 0.5 g of Sulfisoxazole add 2 mL of acetic acid, dissolve by heating with a reflux condenser, add 1 mL of acetic anhydride, and boil for 10 minutes. Add 10 mL of water, cool, alkalize with about 7 mL of a solution of sodium hydroxide (3 in 10), and filter if necessary. Acidify by adding acetic acid dropwise, collect the produced precipitate, recrystalize with methanol, and dry at 105 °C for 1 hour: the crystals melt between 208 and 210 °C.

Melting Point 192 ~ 196 °C (with decomposition).

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Sulfisoxazole in 5 mL of sodium hydroxide TS and 20 mL of water: the solution is clear and colorless to pale yellow.

(2) ***Acid***—To 1.0 g of Sulfisoxazole, add 50 mL of water, warm at 70 °C for 5 minutes, allow to stand in an ice-bath for 1 hour and filter. To 25 mL of the filtrate, add 2 drops of methyl red VS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: a yellow color is observed.

(3) ***Heavy metals***—Proceed with 1.0 g of Sulfisoxazole according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Selenium***—Proceed with 0.2 g of Sulfisoxazole as directed under Oxygen Flask Combustion Method, using 25 mL of diluted nitric acid (1 in 30) as the absorbing liquid. Use a 1 L combustion flask. After combustion, wash the stopper and the inside of the flask with 10 mL of water. Transfer the liquid inside the flask to a 150 mL beaker, using about 20 mL of water. Heat gently to boil, boil for 10 more minutes, cool to room temperature, and use this solution as the test solution. Separately, pipet 6.0 mL of selenium standard stock solution, add 25 mL of diluted nitric acid (1 in 30) and 25 mL of water, and use this solution as the standard solution. Adjust the pH of the test solution and standard solution to 2.0 with diluted ammonia solution (28) (1 in 2), add water to make 60 mL, transfer to a separatory funnel using 10 mL of water, and wash the separatory funnel with 10 mL of water. Add 0.2 g of hydroxylamine hydrochloride, stir to dissolve, add immediately 5.0 mL of 2,3-diaminonaphthalene TS, stopper, mix, and allow to stand at room temperature for 100 minutes. Add 5.0 mL of cyclohexane, shake vigorously for 2 minutes, allow the layers to separate, discard the water layer, centrifuge the cyclohexane extract to remove water, and take the cyclohexane layer. Determine the absorbances at 380 nm of these solutions as directed under Ultraviolet-visible Spectrophotometry, using a solution prepared by adding 25 mL of water to 25 mL of diluted nitric acid (1 in 30) and proceeding in the same manner, as the blank: the absorbance obtained from the test solution is not more than that from the standard solution (not more than 30 ppm).

(5) ***Related substances***—Dissolve 10 mg of Sulfisoxazole in 1 mL of ethyl acetate, and use this solution as the test solution. Separately, dissolve 1 mg of Sulfisoxazole RS in 5 mL of ethyl acetate, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 20 μL each of the test solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone, cyclohexane, and acetic acid (100) (5 : 4 : 1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm and 365 nm): the spot other than the principal spot obtained from the test solution is not larger or more intense than the spot from the standard solution (not more than 2.0 %).

Loss on Drying Not more than 0.5 % (2 g, 105 °C, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 1 g of Sulfisoxazole, previously dried, dissolve in 50 mL of methanol by warming, cool and titrate with 0.2 mol/L sodium hydroxide VS (indicalor: 3 drops of phenolphthalein TS). Perform a blank determination using a mixture of 50 mL of methanol and 18 mL of water and make any necessary correction.

Each mL of 0.2 mol/L sodium hydroxide VS
= 53.46 mg of $C_{11}H_{13}N_3O_3S$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Sulfisoxazole Tablets

Sulfisoxazole Tablets contain not less than 95.0 % and more than 105.0 % of labeled amount of sulfisoxazole ($C_{11}H_{13}N_3O_3S$: 267.30).

Method of Preparation Prepare as directed under Tablets, with Sulfisoxazole.

Identification Extract a portion of powdered Tablets, equivalent to about 1 g of Sulfisoxazole, with 50 mL of ethanol (95) by boiling in a water-bath for 3 minutes, then immediately filter into a beaker. Allow to stand until a quantity of fine, needle-like cystals form. Cool, filter off the crystals, recrystallize from a small volume of ethanol (95) and dry at 105 °C. Determine the infrared spectra of this crystal and the Sulfinpyrazone RS, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Dissolution Test Perform the test with 1 tablet of Sulfisoxazole Tablets at 100 revolution per minute according to Method 1 under the Dissolution Test, using 900 mL of the diluted hydrochloric acid (1 in 12.5) as the dissolution solution. Take 20 mL or more of the dissolved solution after 30 minutes from starting the test and filter through a membrane filter. Discard the first 10 mL of the filtrate, pipet 2.0 mL of the filtrate, add water to make exactly 100 mL and use this solution as the test solution. Separately weigh accurately about 50 mg of Sulfisoxazole RS, dissolve this in dilute hydrochloric acid (1 in 12.5) and make exactly 100 mL. Then, pipet 2.0 mL of this solution, add water to make exactly 100 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 267 nm as directed under Ultraviolet-visible Spectrophotometry.
The dissolution rate of Sulfisoxazole Tablets in 30 minutes is not less than 70 %.

The dissolution rate (%) of sulfisoxazole ($C_{11}H_{13}N_3O_3S$)

$$= W_S \times \frac{A_T}{A_S} \times \frac{1}{C} \times 900$$

W_S: Amount (mg) of Sulfisoxazole RS,
C: Labeled amount (mg) of 1 tablet of Sulfisoxazole Tablets.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Sulfisoxazole Tablets. Weigh accurately a portion of the powder, equivalent to about 20 mg of sulfisoxazole ($C_{11}H_{13}N_3O_3S$), add methanol and shake for 15 minutes, extract, to make exactly 50 mL and filter. Discard the first 10 mL of the filtrate, pipet 5.0 mL of filtrate and 8.0 mL of internal standard solution, add methanol to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately about 20 mg of Sulfisoxazole RS, previously dried at 105 °C for 4 hours, dissolve in methanol to make exactly 50 mL, pipet 5.0 mL of this solution and 8.0 mL of internal standard solution, add methanol to make exactly 50 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculated the ratios, Q_T and Q_S, of the peak area of Sulfisoxazole to that of the internal standard for the test solution and the standard solution, respectively.

Amount (mg) of sulfisoxazole ($C_{11}H_{13}N_3O_3S$)

$$= \text{Amount (mg) of Sulfisoxazole RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—Dissolve 20 mg of Anhydrous Caffeine RS in 50 mL of methanol.

Operating conditions
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column, about 4 mm in internal diameter and about 15 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).
Mobile phase: The mixture of water, methanol and acetic acid (95) (70 : 30 : 1).
Flow rate: Adjust the flow rate so that the retention time of Sulfisoxazole is about 8 minutes.
System suitability
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard and sulfisoxazole are eluted in this order with the resolution between these peaks being not less than 2.0.

Containers and Storage ***Containers***—Well-closed containers.
Storage—Light-resistant.

Sulfur

S: 32.07

[*7704-34-9*]

Sulfur, when dried, contains not less than 99.5 % and not more than 101.0 % of sulfur (S: 32.07).

Description Sulfur appears as pale yellow to yellow powder, and is odorless and tasteless.
Sulfur is freely soluble in carbon disulfide and practically insoluble in water, in ethanol (95) or in ether.

Identification (1) Ignite Sulfur: it burns with a blue flame and gives a pungent odor of sulfur dioxide.

(2) Dissolve 5 mg of Sulfur in 5 mL of sodium hydroxide TS by heating in a water-bath, cool and add 1 drop of sodium pentacyanonitrosylferrate (III) TS: a blue-purple color is observed.

(3) Boil 1 mg of Sulfur with 2 mL of pyridine and 0.2 mL of sodium bicarbonate TS: a blue color is observed.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Sulfur in a mixture of 20 mL of a solution of sodium hydroxide (1 in 6) and 2 mL of ethanol (95) by boiling: the solution is clear. Dissolve 2.0 g of Sulfur in 10 mL of carbon disulfide: the solution is almost clear or slightly opalescent.

(2) ***Acid or alkali***—Shake 2.0 g of Sulfur with 50 mL of freshly boiled and cooled water and add 2 drops of phenolphthalein TS: no red color is observed. Further add 1.0 mL of 0.1 mol/L sodium hydroxide VS: a red color is observed.

(3) ***Arsenic***—Prepare the test solution with 0.20 g of Sulfur according to Method 3 and perform the test (not more than 10 ppm).

Loss on Drying Not more than 1.0 % (1 g, in vacuum, not more than 0.67 kPa, silica gel, 4 hours).

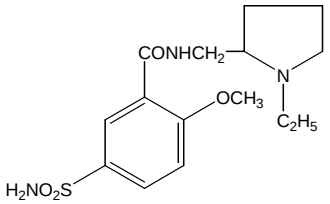
Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 0.4 g of Sulfur, previously dried, dissolve in 20 mL of potassium hydroxide-ethanol TS and 10 mL of water by boiling, cool and add water to make exactly 100 mL. Transfer exactly 25 mL of the solution to a 400-mL beaker, add 50 mL of hydrogen peroxide TS and heat in a water-bath for 1 hour. Acidify the solution with dilute hydrochloric acid, add 200 mL of water, heat to boil, add hot barium chloride TS drop-wise until no more precipitate is produced and heat in a water-bath for 1 hour. Collect the precipitate and wash with water until the last washing shows no opalescence with silver nitrate TS. Dry the precipitate, heat strongly to constant mass and weigh as barium sulfate ($BaSO_4$: 233.39). Perform a blank determination and make any necessary correction.

Amount (mg) of sulfur (S)
= Amount (mg) of barium sulfate ($BaSO_4$) × 0.13739

Containers and Storage ***Containers***—Well-closed containers.

Sulpiride



$C_{15}H_{23}N_3O_4S$: 341.43

N-[(1-ethylpyrrolidin-2-yl)methyl]-2-methoxy-5-sulfamoylbenzamide [*15676-16-1*]

Sulpiride, when dried, contains not less than 98.5 % and not more than 101.0 % of sulpiride ($C_{15}H_{23}N_3O_4S$).

Description Sulpiride is a white, crystalline powder and is odorless.
Sulpiride is freely soluble in acetic acid (100) or in dilute acetic acid, sparingly soluble in methanol, slightly soluble in ethanol (95) or in acetone and practically insoluble in water, in chloroform or in ether.
Sulpiride dissolves in dilute hydrochloric acid or 0.05 mol/L sulfuric acid TS.

Melting point—175 ~ 182 °C (with decomposition).

Identification (1) Dissolve 0.1 g each of Sulpiride and Sulpiride RS in 0.05 mol/L sulfuric acid TS to make 100 mL. Determine the absorption spectra of both solutions, prepared by diluting 5 mL each of the above solutions with water to make 100 mL, as directed under Ultraviolet-visible Spectrophotometry, using water as the blank: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Sulpiride and Sulpiride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity (1) ***Clarity and color of solution***—Dissolve 2.0 g of Sulpiride in 7 mL of dilute acetic acid and add water to make 20 mL: the solution is clear. Perform the test as directed under Ultraviolet-visible Spectrophotometry, using water as the blank: the absorbance at a wavelength of 450 nm does not exceed 0.020.

(2) ***Heavy metals***—Proceed with 2.0 g of Sulpiride as directed under Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Dissolve 50 mg of Sulpiride in 10 mL of methanol and use this solution as the test solution. Dilute 1 mL of the test solution, accurately measured, with methanol to make exactly 100 mL. Dilute 5 mL of this solution, accurately measured, with methanol to make exactly 10 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed

under the Thin-layer Chromatography. Spot 20 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4 : 2 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): spots other than the principal spot from the test solution have no more color than the spot from the standard solution. When the plate is exposed to iodine vapor for 30 minutes, the spots other than the principal spot from the test solution have no more color than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

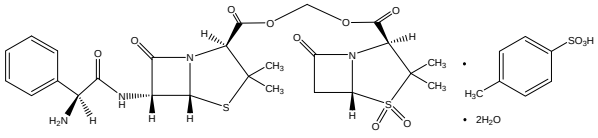
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.4 g of Sulpiride, previously dried, dissolve in 80 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (indicator: 2 drops of methylrosaniline chloride TS). The end point of titration is only when the color of the solution changes from violet through blue to bluish green. Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 34.143 mg of $C_{15}H_{23}N_3O_4S$

Containers and Storage ***Containers***—Well-closed containers.

Sultamicillin Tosilate Hydrate



$C_{25}H_{30}N_4O_9S_2 \cdot C_7H_8O_3S \cdot 2H_2O$: 802.89

(3*S*)-2,2-dimethyl-1,1-dioxo-1λ^6-penam-3-carbonyloxymethyl (3*S*)-2,2-dimethyl-6b-[(2*R*)-2-amimo-2-phenylactamido]penam-3-carboxylate [*83105-70-8*, anhydride]

Sultamicillin Tosilate Hydrate contains not less than 698 μg (potency) and not more than 800 μg (potency) per mg of sultamicillin ($C_{25}H_{30}N_4O_9S_2$: 594.66), calculated on the anhydrous basis and corrected by the amount of residual solvent.

Description Sultamicillin Tosilate Hydrate appears as white to yellowish white crystalline powder.

Sultamicillin Tosilate Hydrate is freely soluble in acetonitrile, in methanol, or in ethanol (99.5) and very slightly soluble in water.

Identification Determine the infrared spectra of Sultamicillin Tosilate Hydrate and Sultamicillin Tosilate RS as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +173 ~ +187° (0.5 g calculated on the anhydrous basis, a mixture of water and acetonitrile (3 : 2), 25 mL, 100 mm)

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Sultamicillin Tosilate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Arsenic***—Proceed with 1.0 g of Sultamicillin Tosilate Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(3) ***Ampicillin***—Perform the procedure rapidly. Weigh accurately about 20 mg of Sultamicillin Tosilate Hydrate, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the test solution. Separately, weigh accurately an amount of Ampicillin RS, equivalent to about 20 mg (potency), and dissolve in the mobile phase to make exactly 100 mL. Pipet 6 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 25 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak area of ampicillin in each solution by the automatic integration method: the peak area from the test solution is not larger than that from the standard solution.

Operating conditions

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: Dissolve 3.12 g of sodium dihydrogen phosphate dihydrate in about 750 mL of water, adjust the pH to 3.0 with diluted phosphoric acid (1 in 10), and add water to make 1000 mL. To 80 mL of acetonitrile for liquid chromatography add this solution to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of ampicillin is about 14 minutes.

System suitability

System performance: Dissolve 12 mg of Ampicillin RS, 4 mg of Sulbactam RS, and 4 mg of *p*-toluenesulfonic acid monohydrate in 1000 mL of the mobile phase. When the procedure is run with 25 μL of this solution under the above operating conditions, sulbactam, *p*-toluenesulfonic acid, and ampicillin are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of ampicillin is not more than 2.0 %.

(4) ***Sulbactam***—Perform the procedure rapidly. Weigh accurately about 20 mg of Sultamicillin Tosilate Hydrate, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the test solution. Separately, weigh accurately an amount of Sulbactam RS, equivalent to about 20 mg (potency), and dissolve in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 25 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak area of sulbactam in each solution by the automatic integration method: the peak area from the test solution is not larger than that from the standard solution.

Operating conditions

Proceed as directed in the operating conditions in the Purity (3).

System suitability

Proceed as directed in the system suitability in the Purity (3).

(5) ***Penicilloic acid***—Weigh accurately about 25 mg of Sultamicillin Tosilate Hydrate, transfer to a stoppered 100 mL flask, dissolve in 1 mL of acetonitrile, and add 25 mL of 0.02 mol/L phosphate buffer solution (pH 3.0). To this solution add exactly 5 mL of 0.005 mol/L iodine VS, stopper tightly, allow to stand for 5 minutes, and titrate with 0.005 mol/L sodium thiosulfate VS (indicator: 1.0 mL of starch TS). Perform a blank determination and make any necessary correction. The amount of penicilloic acid ($C_{25}H_{34}N_4O_{11}S_2$: 630.69) is not more than 3.0 %.

Each mL of 0.005 mol/L sodium thiosulfate VS
= 0.2585 mg of $C_{25}H_{34}N_4O_{11}S_2$

(6) ***Residual solvent***—Weigh accurately about 0.1 g of Sultamicillin Tosilate Hydrate, dissolve in 2 mL of methanol, add water to make exactly 20 mL, and use this solution as the test solution. Separately, weigh accurately about 1 g of ethyl acetate, and add water to make exactly 200 mL. Pipet 2 mL of this solution, add 10 mL of methanol, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of ethyl acetate. Calculate the amount of ethyl acetate by the following equation: not more than 2.0 %.

$$\text{Amount (\%) of ethyl acetate} = \frac{\text{Amount [mg (potency)] of ethyl acetate}}{W_T} \times \frac{A_T}{A_S} \times \frac{1}{5}$$

W_T: Amount (mg) of Sultamicillin Tosilate Hydrate taken

Operating conditions

Detector: A hydrogen flame ionization detector

Column: A column about 3 mm in internal diameter and about 1 m in length, packed with porous styrene-divinylbenzene copolymer for gas chromatography (average pore diameter: 8.5 nm, specific surface area: 300 to 400 m^2/g) (150 to 180 μm in particle diameter)

Column temperature: A constant temperature of about 155 °C

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of ethyl acetate is about 6 minutes.

System suitability

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of ethyl acetate are not less than 500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of ethyl acetate is not more than 5 %.

Water 4.0 ~ 6.0 % (0.5 g, volumetric titration, direct titration)

Residue on Ignition Not more than 0.2 % (1 g)

Assay Perform the procedure rapidly. Weigh accurately about 50 mg (potency) each of Sultamicillin Tosilate Hydrate and Sultamicillin Tosilate RS, and dissolve each in the mobile phase to make exactly 50 mL. To 5 mL each of these solutions add exactly 5 mL of the internal standard solution and the mobile phase to make 25 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of sultamicillin to that of the internal standard.

$$\text{Amount [mg (potency)] of sultamicillin } (C_{25}H_{30}N_4O_9S_2) = \text{Amount [mg (potency)] of Sultamicillin Tosilate RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of isopropyl 4-aminobenzoate in the mobile phase (1 in 2500).

Operating conditions

Detector: An ultraviolet absorption photometer

(wavelength: 215 nm).

Column: A stainless steel column about 3.9 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 35 °C.

Mobile phase: Dissolve 3.12 g of sodium dihydrogen phosphate dihydrate in about 750 mL of water, adjust the pH to 3.0 with diluted phosphoric acid (1 in 10), and add water to make 1000 mL. To 400 mL of acetonitrile for liquid chromatography add this solution to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of sultamicillin is about 4 minutes.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, *p*-toluenesulfonic acid, sultamicillin, and the internal standard are eluted in this order, with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of sultamicilin is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Suxamethonium Chloride for Injection

Suxamethonium Chloride for Injection is a preparation for injection which is reconstituted before use. Suxamethonium Chloride for Injection contains not less than 93.0 % and not more than 107.0 % of labeled amount of suxamethonium chloride ($C_{14}H_{30}Cl_2N_2O_4$: 361.31).

The concentration of Suxamethonium Chloride for Injection is stated as the amount of suxamethonium chloride ($C_{14}H_{30}Cl_2N_2O_4$).

Method for Preparation Prepare as directed under Injections, with Suxamethonium Chloride Hydrate.

Description Suxamethonium Chloride for Injection is a white, crystalline powder or mass.

Identification Weigh an amount of Suxamethonium Chloride for Injection, equivalent to 50 mg of Suxamethonium Chloride Hydrate according to the labeled amount, dissolve in water to make 10 mL and use this solution as the test solution. Separately, dissolve 50 mg of Suxamethonium Chloride Hydrate RS in 10 mL of water and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 1 μL each of the test solution and the standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with the mixture of a solution of ammonium acetate (1 in 100), acetone, 1-butanol and formic acid (20 : 20 : 20 : 1) to a distance of about 10 cm and dry the plate at 105 ºC for 15 minutes. Spray evenly hydrogen hexachloroplatinate (IV)-potassium iodide TS on the plate: the spots from the test solution and the standard solution are blue-purple in color and have similar R_f values.

pH Dissolve 0.1 g of Suxamethonium Chloride for Injection in 10 mL of water: the pH of this solution is between 4.0 and 5.0.

Purity ***Related substances***—Weigh an amount of Suxamethonium Chloride for Injection, equivalent to 0.25 g of Suxamethonium Chloride Hydrate according to the labeled amount and proceed as directed in the Purity (2) under Suxamethonium Chloride Hydrate.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 1.5 EU/mg of suxamethonium chloride.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

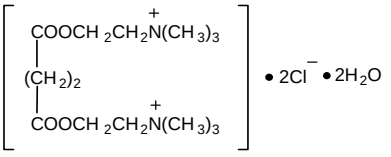
Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately the contents of not less than 10 preparations of Suxamethonium Chloride for Injection. Weigh accurately about 0.5 g of the contents and proceed as directed in the Assay under Suxamethonium Chloride Hydrate.

Each mL of 0.1 mol/L perchloric acid VS
= 18.065 mg of $C_{14}H_{30}Cl_2N_2O_4$

Containers and Storage ***Containers*—**Hermetic containers.

Suxamethonium Chloride Hydrate



$C_{14}H_{30}Cl_2N_2O_4 \cdot 2H_2O$: 397.34

N,*N*,*N*,*N*′,*N*′,*N*′-Hexamethyl-3,8-dioxa-4,7-dioxodecane-1,10-diaminium dichloride dihydrate [*6101-15-1*]

Suxamethonium Chloride Hydrate contains not less than 98.0 % and not more than 101.0 % of suxamethonium chloride ($C_{14}H_{30}Cl_2N_2O_4$: 361.31), calculated on the anhydrous basis.

Description Suxamethonium Chloride Hydrate is a white and crystalline powder.
Suxamethonium Chloride Hydrate is freely soluble in water, in methanol or in acetic acid (100), slightly soluble in ethanol (95), very slightly soluble in acetic anhydride, and practically insoluble in ether.

Identification (1) Determine the infrared spectra of Suxamethonium Chloride Hydrate and Suxamethonium Chloride Hydrate RS, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) A solution of Suxamethonium Chloride Hydrate (1 in 20) responds to the Qualitative Tests for chloride.

Melting Point 159 ~ 164 °C (hydrate form).

pH Dissolve 1.0 g of Suxamethonium Chloride Hydrate in 10 mL of water: the pH of this solution is between 4.0 and 5.0.

Purity (1) ***Clarity and color of solution***— Dissolve 1.0 g of Suxamethonium Chloride Hydrate in 10 mL of water: the solution is clear and colorless.

(2) ***Related substances***—Dissolve 0.25 g of Suxamethonium Chloride Hydrate in 5 mL of water and use this solution as the test solution. Pipet 1 mL of the test solution, add water to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 1 μL each of the test solution and the standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with the mixture of a solution of ammonium acetate (1 in 100), acetone, 1-butanol and formic acid (20 : 20 : 20 : 1) to a distance of about 10 cm and dry the plate at 105 °C for 15 minutes. Spray evenly hydrogen hexachloroplatinate (IV)-potassium iodide TS on the plate and allow to stand for 15 minutes: any spot other than the principal spot from the test solution is not more intense than the spot from the standard solution.

Water 8.0 ~ 10.0 % (0.4 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.4 g of Suxamethonium Chloride Hydrate, dissolve in 80 mL ofthe mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 18.065 mg of $C_{14}H_{30}Cl_2N_2O_4$

Containers and Storage ***Containers***—Tight containers.

Suxamethonium Chloride Injection

Suxamethonium Chloride Injection is an aqueous solution for injection. Suxamethonium Chloride Injection contains not less than 93.0 % and not more than 107.0 % of the labeled amount of suxamethonium chloride ($C_{14}H_{30}Cl_2N_2O_4$: 361.31).
The concentration of Suxamethonium Chloride Injection is stated as the amount of suxamethonium chloride ($C_{14}H_{30}Cl_2N_2O_4$).

Method of Preparation Prepare as directed under Injections, with Suxamethonium Chloride Hydrate.

Description Suxamethonium Chloride Injection is a clear and colorless liquid.

Identification Take a volume of Suxamethonium Chloride Injection, equivalent to 50 mg of Suxamethonium Chloride Hydrate according to the labeled amount, add water to make 10 mL and use this solution as the test solution. Separately, dissolve 50 mg of Suxamethonium Chloride Hydrate RS in 10 mL of water and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 1 μL each of the test solution and the standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of a solution of ammonium acetate (1 in 100), acetone, 1-butanol and formic acid (20 : 20 : 20 : 1) to a distance of about 10 cm and dry the plate at 105 °C for 15 minutes. Spray hydrogen hexachloroplatinate (IV)-potassium iodide TS on the plate: the spots obtained from the test solution and the standard solution are blue-purple in color and have similar R_f.

pH 3.0 ~ 5.0.

Purity ***Hydrolysis products***—Perform the preliminary neutralization with 0.1 mol/L sodium hydroxide VS in the Assay: not more than 0.7 mL of 0.1 mol/L sodium hydroxide VS is required for each 0.2 g of

suxamethonium chloride ($C_{14}H_{30}Cl_2N_2O_4$) taken.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 2.0 EU/mg of suxamethonium chloride.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay Transfer an accurately measured volume of Suxamethonium Chloride Injection, equivalent to about 0.2 g of suxamethonium chloride ($C_{14}H_{30}Cl_2N_2O_4$) to a separatory funnel, add 30 mL of freshly boiled and cooled water and wash the solution with five 20 mL volumes of ether. Combine the ether washings and extract the combined ether layer with two 10 mL volumes of freshly boiled and cooled water. Wash the combined water extracts with two 10 mL volumes of ether. Combine the first solution and the water extracts, add 2 drops of bromthymol blue TS and neutralize with 0.1 mol/L sodium hydroxide VS. Add accurately measured 25 mL of 0.1 mol/L sodium hydroxide VS and boil for 40 minutes under a reflux condenser and cool. Titrate the excess sodium hydroxide with 0.1 mol/L hydrochloric acid VS. Transfer 50 mL of the freshly boiled and cooled water to a flask, add 2 drops of bromthymol blue TS, neutralize the solution with 0.1 mol/L sodium hydroxide VS, perform a blank determination using this solution as the blank and make any necessary correction.

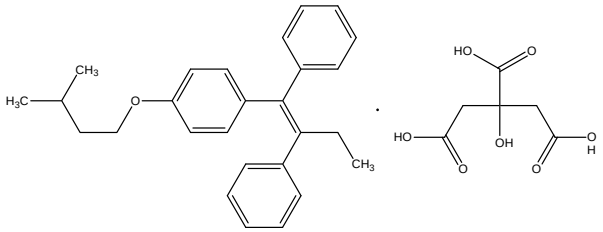
Each mL of 0.1 mol/L sodium hydroxide VS
= 18.065 mg of $C_{14}H_{30}Cl_2N_2O_4$

Containers and Storage ***Containers*—**Hermetic containers.

***Storage*—**Not exceeding 5 °C, and avoid freezing.

Expiration Date 12 months after preparation.

Tamoxifen Citrate



$C_{26}H_{29}NO \cdot C_6H_8O_7$: 563.64

2-[4-[(*Z*)-1,2-Diphenylbut-1-enyl]phenoxy]-*N,N*-dimethylethanamine;2-hydroxypropane-1,2,3-tricarboxylic acid [*54965-24-1*]

Tamoxifen Citrate, when dried, contains not less than 99.0 % and not more than 101.0 % of tamoxifen citrate ($C_{26}H_{29}NO \cdot C_6H_8O_7$).

Description Tamoxifen Citrate appears as a white, fine crystalline powder.
Tamoxifen Citrate is freely soluble in acetic acid (100), sparingly soluble in methanol, and very slightly soluble in water or in ethanol (95).

Melting point—About 142 °C (with decomposition).

Identification (1) Determine the absorption spectra of solutions of Tamoxifen Citrate and Tamoxifen Citrate RS in methanol (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelength.

(2) Determine the infrared spectra of Tamoxifen Citrate and Tamoxifen Citrate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumber.

(3) A solution of Tamoxifen Citrate (1 in 100) responds to the Qualitative Tests for citrate.

Purity (1) ***Heavy metals*—**Proceed with 1.0 g of Tamoxifen Citrate according to Method 2 and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Iron*—**Proceed with 1.0 g of Tamoxifen Citrate according to Method 3, and use this solution as the test solution. Separately, proceed with 5.0 mL of standard Iron solution according to the procedure of the test solution, and use this solution as the control solution. Perform the test with the test solution and the control solution according to method A: the test solution is not more intense than the control solution (not more than 50 ppm).

(3) ***E-isomers*—**Weigh accurately about 30 mg of Tamoxifen, dissolve in the mobile phase to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately about 30 mg of Tamoxifen Citrate RS, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 20 µL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine peak areas, A_T and A_S, of *E*-isomers as citrate for the test solution and the standard solution, respectively (not more than 0.3 %).

$$\text{Amount (mg) of E-isomers } (C_{26}H_{29}NO \cdot C_6H_8O_7) = 0.05 \times \frac{A_T}{A_S}$$

C : Concentration (μg/mL) of E-isomer as citrate ($C_{26}H_{29}NO \cdot C_6H_8O_7$) based on its delared content in Tamoxifen Citrate RS in the standard solution.

Operating conditions

Detector : An ultraviolet absorption photometer (wavelength: 254 nm).

Column : A stainless steel column, about 4.0 mm in internal diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Mobile phase : Dissolve 2 mL of acetic acid (100) and 1.08 g of sodium 1-octanesulfonic acid in 320 mL of water, and add methanol to make 1000 mL.

Flow rate : 0.7 mL/minute

System suitability

System performance : When the procedure is run with 20 μL of the standard solution according to the above operating conditions, the relative retention time of E-isomer peak to the retention time of *Z*-isomer is not more than 0.93.

System repeatability : When the test is repeated 5 times with 20 μL each of the standard solution according to the above operating conditions, the relative standard deviation of the peak area of Tamoxifen Citrate is not more than 3.0 %.

(4) ***Related substances*—**Conduct this procedure rapidly, using light-resistant vessels. Weigh accurately about 15 mg of Tamoxifen Citrate, dissolve in the mobile phase to make 10 mL, and use this solution as the test solution. Pipet 1 mL of the test solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method: the area of the peak other than tamoxifen from the test solution is not larger than 3/10 times the peak area of tamoxifen from the standard solution, and the total area of the peaks other than tamoxifen from the test solution is not larger than 4/5 times the peak area of tamoxifen from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 240 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column: A constant temperature of about 25 °C

Mobile phase: Dissolve 4.8 g of *N,N*-dimethyl-*n*-octylamine in 1000 mL of water. Separately, dissolve 0.9 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water. Mix these solutions, and adjust the pH to 3.0 with phosphoric acid. To 600 mL of this solution add 400 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of tamoxifen is about 21 minutes.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make 10 mL. Confirm that the peak area of tamoxifen obtained from 10 μL of this solution is equivalent to 8 to 12 % of that from the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of tamoxifen are not less than 5000 and not more than 1.5, repectively.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tamoxifen is not more than 1.5 %.

Time span of measurement: About 2.5 times as long as the retention time of tamoxifen, beginning after the solvent peak.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

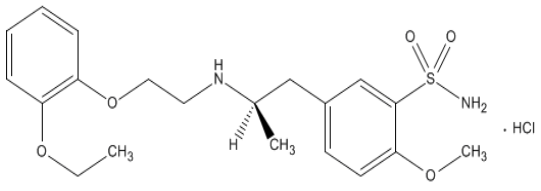
Assay Weigh accurately about 1.0 g of Tamoxifen Citrate, and dissolve in 150 mL of acetic acid (100), and titrate the solution with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1mol/L perchloric acid VS
= 56.36 mg of $C_{26}H_{29}NO \cdot C_6H_8O$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Tamsulosin Hydrochloride



$C_{20}H_{28}N_2O_5S \cdot HCl$: 444.97

5-[(2*R*)-2-[2-(2-Ethoxyphenoxy)ethylamino]propyl]-2-methoxybenzenesulfonamidehydrochloride
[*106463-17-6*]

Tamsulosin Hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S \cdot HCl$).

Description Tamsulosin Hydrochloride appears as white crystals.
Tamsulosin Hydrochloride is freely soluble in formic acid, sparinlyl soluble in water, slightly soluble in acetic acid (100), and very slightly soluble in ethanol (99.5).

Melting point—About 230 °C (with decomposition).

Identification (1) Determine the absorption spectra of solutions of Tamsulosin Hydrochloride and Tamsulosin Hydrochloride RS (3 in 160000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Tamsulosin Hydrochloride and Tamsulosin Hydrochloride RS as directed in the potassium chloride disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 5 mL of an ice-cooled solution of Tamsulosin Hydrochloride (3 in 400) add 3 mL of dilute nitric acid, shake well, allow to stand for 30 minutes, and filter: the filtrate responds to the Qualitative Tests for chloride.

Specific Optical Rotation $[\alpha]_D^{20}$: -17.5 ~ -20.5° (0.15 g after drying, water, warming, after cooling, 20 mL, 100 mm).

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Tamsulosin Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—(i) Dissolve 50 mg of Tamsulosin Hydrochloride in 10 mL of the mobile phase, and use this solution as the test solution. Pipet 2 mL of this solution, add the mobile phase to make exactly 50 mL, pipet 2.5 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas of each solution by the automatic integration method: the total area of the peaks other than tamsulosin from the test solution is not larger than the peak area of tamsulosin from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 225 nm)

Column: A stainless steel column about 4.0 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: Dissolve 4.4 mL of perchloric acid and 1.5 g of sodium hydroxide in 950 mL of water, adjust the pH to 2.0 with sodium hydroxide TS, and add water to make 1000 mL. To 700 mL of this solution add 300 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of tamsulosin is about 6 minutes.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of tamsulosin obtained from 10 μL of this solution is equivalent to 1.4 to 2.6 % of that from the standard solution.

System performance: Dissolve 5 mg of tamsulosin hydrochloride and 10 mg of propyl paraoxybenzoate in 20 mL of the mobile phase. To 2 mL of this solution add the mobile phase to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, tamsulosin and propyl paraoxybenzoate are eluted in this order with the resolution between these peaks being not less than 12.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tamsulosin is not more than 4.0 %.

Time span of measurement: Until tamsulosin is eluted (excluding the solvent peak).

(ii) Perform the test with 10 μL each of the test solution and standard solution obtained from Related substances (i) as directed under Liquid Chromatography according to the following conditions. Determine the peak areas of each solution by the automatic integration method: the total area of the peaks other than tamsulosin from the test solution is not larger than the peak area of tamsulosin from the standard solution.

Operating conditions

Detector, column, and column temperature: Proceed as directed in the operating conditions in Related substances (i).

Mobile phase: Dissolve 4.4 mL of perchloric acid and 1.5 g of sodium hydroxide in 950 mL of water, adjust the pH to 2.0 with sodium hydroxide TS, and add water to make 1000 mL. To this solution add 1000 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of tamsulosin is about 2.5 minutes.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase from Related substances (i) to make exactly 50 mL. Confirm that the peak area of tamsulosin obtained from 10 μL of this solution is equivalent to 1.4 to 2.6 % of that from the standard solution.

System performance: Proceed as directed in the system performance in Related substances (i).

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tamsulosin is not more than 4.0 %.

Time span of measurement: About 5 times as long as the retention time of tamsulosin, beginning after the peak of tamsulosin.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.7 g of Tamsulosin Hydrochloride, previously dried, dissolve in 5 mL of formic acid, add 75 mL of a mixture of acetic acid (100) and acetic anhydride (3 : 2), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 44.50 mg of $C_{20}H_{28}N_2O_5S \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Tannic Acid

Tannin [*1401-55-4*]

Tannic Acid is the tannin usually obtained from nutgalls or rhusgalls.

Description Tannic Acid is a yellowish white to pale brown, amorphous powder, glistening leaflets, or spongy masses, is odorless or has a faint, characteristic odor and has a strongly astringent taste.
Tannic Acid is very soluble in water or in ethanol (95) and practically insoluble in ether.

Identification (1) To 5 mL of a solution of Tannic Acid (1 in 400), add 2 drops of iron (III) chloride TS: a blue-black color is observed. Allow the solution to stand: a blue-black precipitate is produced.

(2) To 5 mL of a solution of Tannic Acid (1 in 20), add 1 drop each of albumin TS, gelatin TS, or 1 mL of starch TS: a precipitate is produced in each solution.

Purity (1) ***Gum, dextrin and sucrose***—Dissolve 3.0 g of Tannic Acid in 15 mL of boiling water: the solution is clear or slightly turbid. Cool and fiter the solution. To 5 mL of the filtrate, add 5 mL of ethanol (95): no turbidity is produced, add further 3 mL of ether to this solution: no turbidity is produced.

(2) ***Resinous substances***—To 5 mL of the filtrate obtained in (1), add 10 mL of water: no turbidity is produced.

(3) ***Heavy metals***—Proceed with 0.5 g of Tannic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 40 ppm).

(4) ***Arsenic***—Prepre the test solution with 0.67 g of Tannic Acid according to Method 2, and perform the test (not more than 3 ppm).

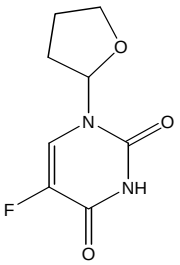
Loss on Drying Not more than 12.0 % (1 g, 105 °C, 2 hours).

Residue on Ignition Not more than 1.0 % (0.5 g).

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Tegafur



and enantiomer

$C_8H_9FN_2O_3$: 200.17

(*RS*)-5-Fluoro-1-(tetrahydrofuran-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione [*17902-23-7*]

Tegafur, when dried, contains not less than 98.0 % and not more than 101.0 % of tegafur ($C_8H_9FN_2O_3$).

Description Tegafur is a white, crystalline powder.
Tegafur is soluble in methanol or in acetone, sparingly soluble in water or in ethanol (95).
Tegafur dissolves in dilute sodium hydroxide TS.
A solution of Tegafur in methanol (1 in 50) shows no optical rotation.

Identification (1) Determine the absorption spectra of solutions of Tegafur and Tegafur RS in 0.01 mol/L sodium hydroxide TS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Tegafur and Tegafur RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Prepare the test solution with 10 mg of Tegafur as directed under Oxygen Flask Combustion Method, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as an absorbing liquid:

the test solution responds to the Qualitative Tests (2) for fluoride.

Melting Point 166 ~ 171 °C.

pH Dissolve 0.5 g of Tegafur in 50 mL of water: the pH of this solution is between 4.2 and 5.2.

Purity (1) ***Clarity and color of solution*—**Dissolve 0.2 g of Tegafur in 10 mL of dilute sodium hydroxide TS: the solution is clear and colorless.

(2) ***Chloride*—**Dissolve 0.8 g of Tegafur in 40 mL of water by warming, cool, filter, if necessary and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011 %).

(3) ***Heavy metals*—**Dissolve 1.0 g of Tegafur in 40 mL of water by warming, cool, filter, if necessary and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(4) ***Arsenic*—**To 1.0 g of Tegafur in a crucible, add 10 mL of a solution of magnesium nitrate in ethanol (1 in 10), fire the ethanol (95) to burn and incinerate by ignition between 750 °C and 850 °C. If a carbonized substance remains, moisten with a small quantity of nitric acid and ignite again to incinerate. After cooling, dissolve the residue in 10 mL of dilute hydrochloric acid by warming in a water-bath and perform the test with this solution as the test solution (not more than 2 ppm).

(5) ***Related substances*—**Dissolve 0.1 g of Tegafur in 10 mL of methanol and use this solution as the test solution. Pipet 1.0 mL of the test solution, add methanol to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (95) (5 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 4 hours).

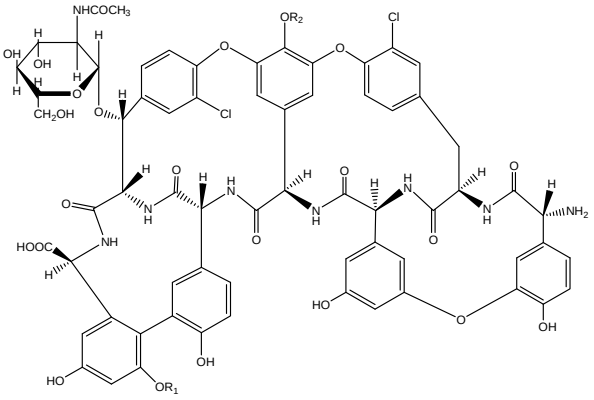
Residue on Ignition Not more than 0.1 % (1 g, platinum crucible).

Assay Weigh accurately about 0.15 g of Tegafur, previously dried, place in an iodine bottle, dissolve in 75 mL of water and add exactly 25 mL of 1/60 mol/L potassium bromate VS. Add rapidly 1.0 g of potassium bromide and 12 mL of hydrochloric acid, stopper the bottle tightly at once and allow to stand for 30 minutes with occasional shaking. To this solution, add 1.6 g of potassium iodide, shake gently, allow to stand for exactly 5 minutes and titrate the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS). Perform a blank determination and make any necessary correction.

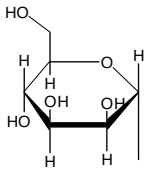
Each mL of 1/60 mol/L potassium bromate VS
= 10.008 mg of $C_8H_9FN_2O_3$

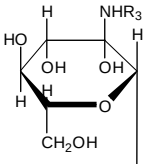
Containers and Storage ***Containers***—Tight containers.

Teicoplanin



R_1 =





Teicoplanin A_2 group: R_2 =

Teicoplanin $A_{2\text{-}1}$
$R_3 = COCH_2CH_2CH{=}CHCH_2CH_2CH_2CH_2CH_3$
Teicoplanin $A_{2\text{-}2}$
$R_3 = COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH(CH_3)_2$
Teicoplanin $A_{2\text{-}3}$
$R_3 = COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_3$
Teicoplanin $A_{2\text{-}4}$
$R_3 = COCH_2CH_2CH_2CH_2CH_2CH(CH_3)CH_2CH_3$
Teicoplanin $A_{2\text{-}5}$
$R_3 = COCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH(CH_3)_2$
Teicoplanin A_3
R_2 = H

Teicoplanin $A_{2\text{-}1}$ $C_{88}H_{95}Cl_2N_9O_{33}$: 1877.64
(3*S*,15*R*,18*R*,34*R*,35*S*,38*S*,48*R*,50a*R*)-34-(2-Acetylamino-2-deoxy-*β*-D-glucopyranosyloxy)-15-

amino-22,31-dichloro-56-[2(Z)-dec-4-enoylamino-2-deoxy-β-D-glucopyranosyloxy]-2,3,16,17,18,19,35,37,36,38,48,49,50,50a-tetradecahydro-6,11,40,44-tetrahydroxy-42-(α-D-mannopyranosyloxy)-2,16,36,50,51,59,-hexaoxo-1H,15H,34H-20,23:30.33-dietheno-3,18:35,48-bis(iminomethano)-4,8:10,14:25,28:43,47-tetrametheno-28H-[1,14,6,22]dioxadiazacyclooctacosino[4,5-m][10,2,16]benzoxadiazacyclotetracosine-38-carboxylic acid [*91032-34-7*]

Teicoplanin A_{2-2} $C_{88}H_{97}Cl_2N_9O_{33}$: 1879.66
(3*S*,15*R*,18*R*,34*R*,35*S*,38*S*,48*R*,50a*R*)-34-(2-Acetylamino-2-deoxy-β-D-glucopyranosyloxy)-15-amino-22,31-dichloro-56-[2-deoxy-2(8-metylnonanoylamino)-β-D-glucopyranosyloxy]-2,3,16,17,18,19,35,36,37,38,48,49,50,50a-tetradecahydro-6,11,40,44-tetrahydroxy-42-(α-D-mannopyranosyloxy)-2,16,36,50,51,59,-hexaoxo-1*H*,15*H*,34*H*-20,23:30.33-dietheno-3,18:35,48-*bis*(iminomethano)-4,8:10,14:25,28:43,47-tetrametheno-28*H*-[1,14,6,22]dioxadiazacyclooctacosino[4,5-*m*][10,2,16]benzoxadiazacyclotetracosine-38-carboxylic acid [*91032-26-7*]

Teicoplanin A_{2-3} $C_{88}H_{97}Cl_2N_9O_{33}$: 1879.66
(3*S*,15*R*,18*R*,34*R*,35*S*,38*S*,48*R*,50a*R*)-34-(2-Acetylamino-2-deoxy-β-D-glucopyranosyloxy)-15-amino-22,31-dichloro-56-[2-decanoylamino-2-deoxy-β-D-glucopyranosyloxy]-2,3,16,17,18,19,35,36,37,38,48,49,50,50a-tetradecahydro-6,11,40,44-tetrahydroxy-42-(α-D-mannopyranosyloxy)-2,16,36,50,51,59,-hexaoxo-1*H*,15*H*,34*H*-20,23:30.33-dietheno-3,18:35,48-bis(iminomethano)-4,8:10,14:25,28:43,47-tetrametheno-28*H*-[1,14,6,22]dioxadiazacyclooctacosino[4,5-*m*][10,2,16]benzoxadiazacyclotetracosine-38-carboxylic acid [*91032-36-9*]

Teicoplanin A_{2-4} $C_{89}H_{99}Cl_2N_9O_{33}$: 1893.68
(3*S*,15*R*,18*R*,34*R*,35*S*,38*S*,48*R*,50a*R*)-34-(2-Acetylamino-2-deoxy-β-D-glucopyranosyloxy)-15-amino-22,31-dichloro-56-[2-deoxy-2-(8-methyldecanoylamino-β-D-glucopyranosyloxy]-2,3,16,17,18,19,35,36,37,38,48,49,50,50a-tetradecahydro-6,11,40,44-tetrahydroxy-42-(α-D-mannopyranosyloxy)-2,16,36,50,51,59,-hexaoxo-1*H*,15*H*,34*H*-20,23:30.33-dietheno-3,18:35,48-*bis*(iminomethano)-4,8:10,14:25,28:43,47-tetrametheno-28*H*-[1,14,6,22]dioxadiazacyclooctacosino[4,5-m][10,2,16]benzoxadiazacyclotetracosine-38-carboxylic acid [*91032-37-0*]

Teicoplanin A_{2-5} $C_{89}H_{99}Cl_2N_9O_{33}$: 1893.68
(3*S*,15*R*,18*R*,34*R*,35*S*,38*S*,48*R*,50a*R*)-34-(2-Acetylamino-2-deoxy-β-D-glucopyranosyloxy)-15-amino-22,31-dichloro-56-[2-deoxy-2-(9-methyldecanoylamino-β-D-glucopyranosyloxy]-2,3,16,17,18,19,35,36,37,38,48,49,50,50a-tetradecahydro-6,11,40,44-tetrahydroxy-42-(α-D-mannopyranosyloxy)-2,16,36,50,51,59,-hexaoxo-1*H*,15*H*,34*H*-20,23:30.33-dietheno-3,18:35,48-bis(iminomethano)-4,8:10,14:25,28:43,47-tetrametheno-28*H*-[1,14,6,22]dioxadiazacyclooctacosino[4,5-*m*][10,2,16]benzoxadiazacyclotetracosine-38-carboxylic acid [*91032-38-1*]

Teicoplanin A_{3-1} $C_{72}H_{68}Cl_2N_8O_{28}$: 1564.25
(3*S*,15*R*,18*R*,34*R*,35*S*,38*S*,48*R*,50a*R*)-34-(2-Acetylamino-2-deoxy-β-D-glucopyranosyloxy)-15-amino-22,31-dichloro-2,3,16,17,18,19,35,36,37,38,48,49,50,50a-tetradecahydro-6,11,40,44-tetrahydroxy-42-(α-D-mannopyranosyloxy)-2,16,36,50,51,59,-hexaoxo-1*H*,15*H*,34*H*-20,23:30.33-dietheno-3,18:35,48-bis(iminomethano)-4,8:10,14:25,28:43,47-tetrametheno-28*H*-[1,14,6,22]dioxadiazacyclooctacosino[4,5-*m*][10,2,16]benzoxadiazacyclotetracosine-38-carboxylic acid [*93616-27-4*]

[*61036-62-2*, Teicoplanin]

Teicoplanin is a mixture of glycopeptides substances having antibacterial activity produced by the growth of *Actinoplanes teichomyceticus*.
Teicoplanin contains not less than 900 μg (potency) per mg of teicoplanin ($C_{72\sim89}H_{68\sim99}Cl_2N_{8\sim9}O_{28\sim33}$), calculated on the anhydrous, de-sodium chloride, and de-residual solvents basis.

Description Teicoplanin appears as white to pale yellowish white powder.
Teicoplanini s freely soluble in water, sparingly soluble in *N,N*-dimethylformamide, and practically insoluble in acetonitrile, in methanol, in ethanol (95), in acetone, in acetic acid (100), or in ether.

Identification (1) To 1 mL of a solution of Teicoplanin (1 in 100) add 2 mL of ninhydrin TS, and warm for 5 minutes: a blue-purple color develops.

(2) To 1 mL of a solution of Teicoplanin (3 in 100) add slowly 2 mL of anthrone TS, and shake gently: a dark brown color develops.

(3) Determine the infrared spectra of Teicoplanin and Teicoplanin RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH Dissolve 0.5 g of Teicoplanin in 10 mL of water: the pH of the solution is between 6.3 and 7.7.

Content Ratio Dissolve about 20 mg of Teicoplanin in water to make 10 mL, and use this solution as the

test solution. Perform the test with 20 μL fo the test solution as directed under Liquid Chromatography according to the following conditions, and calculate the sum of the peak areas of the teicoplanin A_2 group, S_a, the sum of the peak areas of the teicoplanin A_3 group, S_b, and the sum of the peak areas of the other contents, S_c, by the automatic integration method. Calculate each amount by the following equations: the teicoplanin A_2 group is not less than 80.0 %, the teicoplanin A_3 group is not more than 15.0 %, and the other contents are not more than 5.0 %. The elution order of each content of teicoplanin and the relative retention times of each content with respect to teicoplanin $A_{2\text{-}2}$ are as follows.

Name of content	Elution order	Relative retention time
Teicoplanin A_3 group		≤0.42
Teicoplanin $A_{3\text{-}1}$	1	0.29
Teicoplanin A_2 group		0.42<, ≤1.25
Teicoplanin $A_{2\text{-}1}$	2	0.91
Teicoplanin $A_{2\text{-}2}$	3	1.00
Teicoplanin $A_{2\text{-}3}$	4	1.04
Teicoplanin $A_{2\text{-}4}$	5	1.17
Teicoplanin $A_{2\text{-}5}$	6	1.20
Other contents		1.25<

$$\text{Amount (\%) of the teicoplanin } A_2 \text{ group} = \frac{S_a}{S_a + 0.83S_b + S_c} \times 100$$

$$\text{Amount (\%) of the teicoplanin } A_3 \text{ group} = \frac{0.83S_b}{S_a + 0.83S_b + S_c} \times 100$$

$$\text{Amount (\%) of the other contents} = \frac{S_c}{S_a + 0.83S_b + S_c} \times 100$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in 1650 mL of water, add 300 mL of acetonitrile, adjust the pH to 6.0 with sodium hydroxide TS, and add water to make 2000 mL.

Mobile phase B: Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in 550 mL of water, add 1400 mL of acetonitrile, adjust the pH to 6.0 with sodium hydroxide TS, and add water to make 2000 mL.

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-32	100→70	0→30
32-40	70→50	30→50
40-42	50→100	50→0

Flow rate: 1.8 mL/minute

Time span of measurement: About 1.7 times as long as the retention time of teicoplanin $A_{2\text{-}2}$, beginning after the solvent peak.

System suitability

Test for required detectability: Confirm that the peak height of teicoplanin $A_{2\text{-}2}$ obtained from the test solution is equivalent to about 90 % of the full scale.

System performance: When the procedure is run with 20 μL of the test solution under the above operating conditions, the symmetry factor of the peak of teicoplanin $A_{3\text{-}1}$ is not more than 2.2.

System repeatability: When the test is repeated 3 times with 20 μL each of the test solution under the above operating conditions, the relative standard deviation of the peak area of teicoplanin $A_{2\text{-}2}$ is not more than 2.0 %.

Purity (1) ***Sodium chloride***—Weigh accurately about 0.5 g of Teicoplanin, dissolve in 50 mL of water, titrate with 0.1 mol/L silver nitrate VS (indicator: 1 mL of potassium chromate TS), and calculate the amount of sodium chloride: not more than 5.0 %.

Each mL of 0.1 mol/L silver nitrate VS
= 5.844 mg of NaCl

(2) ***Heavy metals***—Proceed with 1.0 g of Teicoplanin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Residual solvents***—Weigh accurately about 0.1 g of Teicoplanin, dissolve in *N,N*-dimethylformamide to make exactly 10 mL, and use this solution as the test solution. Separately, weigh accurately about 1 g each of methanol and acetone, and add *N,N*-dimethylformamide to make exactly 100 mL. Pipet 1 mL of this solution, add *N,N*-dimethylformamide to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 4 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following conditions, determine the peak areas, A_1 and A_2, of methanol and acetone in the test solution, and the peak areas, A_{S1} and A_{S2}, of methanol and acetone in the standard solution, and calculate the amount of methanol and acetone in Teicoplanin by the following equations: not more than 0.5 % and not more than 1.0 %, respectively.

$$\text{Amount (\%) of methanol} = W_{S1} \times \frac{A_1}{A_{S1}} \times 0.001 \times \frac{1}{W_{T1}} \times 100$$

W_{S1}: Amount (g) of methanol taken
W_{T1}: Amount (g) of Teicoplanin taken

$$\text{Amount (\%) of acetone} = W_{S2} \times \frac{A_2}{A_{S2}} \times 0.001 \times \frac{1}{W_{T2}} \times 100$$

W_{S2}: Amount (g) of acetone taken
W_{T2}: Amount (g) of Teicoplanin taken

Operating conditions

Detector: A hydrogen flame ionization detector

Column: A gas column 2 mm in internal diameter and 3 m in length, packed with graphite carbon for gas chromatography (150 to 180 μm in particle diameter), coated with esterified polyethylene glycol for gas chromatography at the rate of 0.1 %.

Column temperature: Maintain at 70 °C for 4 minutes, then raise the temperature to 210 °C at the rate of 8 °C per minute.

Detector temperature: A constant temperature of about 240 °C

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention times of methanol and acetone are about 2 minutes and about 5 minutes, respectively.

System suitability

Test for required detectability: Confirm that the peak height of acetone obtained from 4 μL of the standard solution is equivalent to about the full scale.

System performance: When the procedure is run with 4 μL of the standard solution under the above operating conditions, methanol and acetone are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 3 times with 4 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of acetone is not more than 3.0 %.

Water Not more than 15.0 % (0.2 g, volumetric titration, direct titration).

Sterility Test It meets the requirement, when Teicoplanin is used in a sterile preparation.

Bacterial Endotoxins Less than 0.75 EU/mg (potency) of teicoplanin, when Teicoplanin is used in a sterile preparation.

Assay ***The Cylinder-plate method*** (1) Test organism- *Bacillus subtilis* ATCC 6633

(2) Agar media for seed and base layer- Use the culture medium in I 2 1) (1) ① under Microbial Assay for Antibiotics.

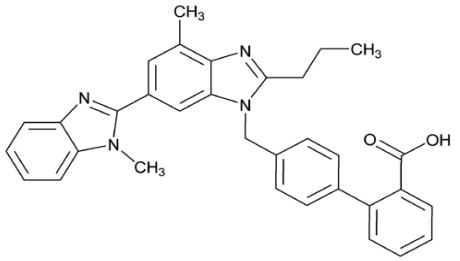
(3) Standard solutions- Weigh accurately about 50 mg (potency) of Teicoplanin RS, dissolve in phosphate buffer solution (pH 6.0) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 5 °C, and use within 14 days. Pipet a suitable volume of the standard stock solution, add the phosphate buffer solution (pH 6.0) so that each mL contains 160 μg (potency) and 40 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(4) Test solutions- Weigh accurately about 50 mg (potency) of Teicoplanin, and dissolve in pH 6.0 phosphate buffer solution to make exactly 50 mL. Pipet a suitable volume of this solution, add phosphate buffer solution (pH 6.0) so that each mL contains 160 μg (potency) and 40 μg (potency), and use these solutions as the high concentration test solution and low concentration test solution, respectively. Perform the test with these solutions as directed in I 8 under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and not exceeding 5 °C.

Telmisartan



$C_{33}H_{30}N_4O_2$: 514.62

2-(4-{[4-Methyl-6-(1-methyl-1*H*-1,3-benzodiazol-2-yl)-2-propyl-1*H*-1,3-benzodiazol-1-yl]methyl}phenyl) benzoic acid [*144701-48-4*]

Telmisartan, when dried, contains not less than 98.0 % and not more than 101.0 % of telmisartan ($C_{33}H_{30}N_4O_2$).

Description Telmisartan appears as white to pale yellow crystalline powder.

Telmisartan is sparingly soluble in dichloromethane, slightly soluble in methanol, and practically insoluble in water.

Telmisartan dissolves in 1 mol/L sodium hydroxide.

Identification (1) Determine the infrared spectra of Telmisartan and Telmisartan RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers. If the two spectra are different, dissolve Telmisartan and Telmisartan RS in ethanol (95), and heat if necessary. Cool this solution in ice water, filter the precipitate, dry at 105 °C, and repeat the determination.

(2) The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Telmisartan according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Weigh accurately about 100 mg of Telmisartan, add 20 mL of methanol and 100 μL of 1 mol/L sodium hydroxide TS, sonicate to dissolve completely, add methanol to make 40 mL, and use this solution as the test solution. Protect the test solution from light after preparation and use immediately. Separately, weigh accurately 100 mg of Telmisartan RS, add 20 mL of methanol and 100 μL of 1 mol/L sodium hydroxide, sonicate to dissolve completely, and add methanol to make 40 mL. To 1 mL of this solution add methanol to make 100 mL, and use this solution as the standard solution. Perform the test with 2 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method. Calculate the amount of related substances in the test solution: the amounts of telmisartan related substance I {1,7-dimethyl-2-propyl-1*H*,3*H*-2,5-bibenzo[*d*]imidazole}, telmisartan amide, telmisartan related substance II {4-[(1,7-dimethyl-2-propyll-1*H*,1*H*-2,5-bibenzo[*d*]imidazole-1-yl)methyl]biphenyl-2-carboxylic acid}, and telmisartan diacid, having the relative retention times of about 0.3, about 0.7, about 0.9, and about 1.1 with respect to telmisartan, are not more than 0.1 %, respectively. The amounts of telmisartan *t*-butyl ester and telmisartan unknown related substance, having the relative retention times of about 1.7 and about 1.8, are not more than 0.2 %, respectively. Any other related substance is not more than 0.1 %, and the total amount of related substances is not more than 1.0 %. Disregard any related substance of not more than 0.05 %.

$$\text{Amount (\%) of each related substance} = \frac{A_i}{A_S} \times \frac{C_S}{C_T} \times 100$$

A_i: Peak area of each related substance from the test solution

A_S: Peak area of each related substance from the standard solution

C_S: Concentration of Telmisartan RS in the standard solution

C_T: Concentration of telmisartan in the test solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm)

Column: A stainless steel column about 4.0 mm in internal diameter and about 12.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: Dissolve 2.0 g of potassium dihydrogen phosphate and 3.8 g of sodium 1-pentanesulfonate in 1000 mL of water, and adjust the pH to 3.0 with phosphoric acid.

Mobile phase B: A mixture of acetonitrile and methanol (4 : 1)

Column temperature: A constant temperature of about 40 °C

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-2	70	30
2-27	70→20	30→80
27-32	20	80
32-32.1	70	30
32.1-37	70	30

Flow rate: 1 mL/minute

System suitability

System performance: Weigh accurately 100 mg of Telmisartan RS and 100 mg of Telmisartan Related Substance II RS, add 20 mL of methanol and 100 μL of 1 mol/L sodium hydroxide, sonicate to dissolve completely, add methanol to make 40 mL, and use this solution as the system suitability solution. When the procedure is run with 2 μL of the system suitability solution under the above operating conditions, the resolution between the peaks of telmisartan and telmisartan related substance II is not less than 3.0, and the symmetry factor of telmisartan related substance II is 0.9 to 1.5.

System repeatability: When the test is repeated 6 times with 2 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of telmisartan is not more than 5.0 %.

Loss on Drying Not more than 1.5 % (1 g, 105 °C).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 190 mg of Telmisartan, dissolve in 5 mL of anhydrous formic acid, add 75 mL of acetic anhydride, and titrate with

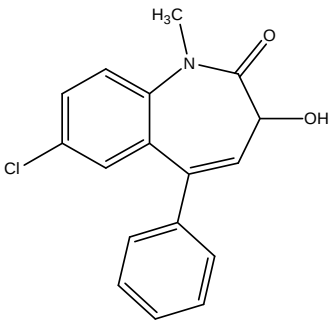
0.1 mol/L perchloric acid VS. Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 25.73 mg of $C_{33}H_{30}N_4O_2$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Temazepam



$C_{16}H_{13}O_2N_2Cl$: 300.74

7-Chloro-3-hydroxy-1-methyl-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-2-one [*846-50-4*]

Temazepam containes not less than 98.0 % not more than 102.0 % of temazepam ($C_{16}H_{13}O_2N_2Cl$), calculated on the dried basis.

Description Temazepam is a white, crystalline powder.

Temazepam is sparingly soluble in ethanol (95) and very slightly soluble in water.

Melting point —157 ~ 163 °C.

Identification (1) Determine the absorption spectra of solutions of Temazepam and Temazepam RS in methanol (1 in 80000) as directed under Ultraviolet-visible Spectrophotometry: both specra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Temazepam and Temazepam RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibits similar intensities of absorption at the same wavenumbers.

(3) The retention time of the principal peak obtained from the test solution in the Assay corresponds to the retention time of the principal peak obtained from the standard solution.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Temazepam according to Method 2 and perform the test. Prepare the control solution with 2 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Weigh exactly about 0.1 g of Temazepam, dissolve in chloroform to make exactly 10 mL, and use this solution as the test solution. Weigh exactly 0.1 g of Temazepam RS, dissolve in chloroform to make exactly 10 mL. Pipet 1.0 mL of this solution, add chloroform to make each 100 mL and 200 mL, and use these solutions as the standard solution (1) and the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography. Spot each 10 μL of the test solution, the standard solution (1) and the standard solution (2) on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, chloroform, methanol and ammonia solution (28) (50:40:12:1) to a distance of about 10 cm and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm), and compare the intensities of any secondary spots observed in the chromatograms of the test solution with those of the principal spots in the chromatograms of the standard solutions: the spots other than the principal spot obtained from the test solution are not darker than the principal spot obtained from the standard solution (1) (1.0 %), and the sum of the intensities of the spots other than the principal spot obtained from the test solution is not greater than 4 times of intensity of the spot obtained from the standard solution (2) (2.0 %).

Loss on Drying not more than 0.5 % (1 g, 105 °C, 2 hours).

Residue on Ignition not more than 0.1 % (1 g).

Assay Weigh acculately about 40 mg each Temazepam and Temazepam RS, dissolve in the diluent to make exactly 200 mL and use these solutions as the test solution and the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine the peak areas, A_T and A_S, of temazepam in each solution.

$$\text{Content (\%) of temazepam } (C_{16}H_{13}O_2N_2Cl) = \frac{A_T}{A_S} \times \frac{C_S}{C_T} \times 100$$

C_S: Concentration (mg/mL) of the standard solution
C_T: Concentration (mg/mL) of the test solution

Diluent—A mixture of methanol and water (90 : 10)

Operating conditions

Detector : An ultraviolet absorption photometer (wavelength : 254 nm).

Column : A stainless steel column, about 4 mm in internal diameter and about 25 cm in length, packed with dimethylsilanized silica gel for the liquid chromatography (5 μm in particle diameter).

Mobile phase : A mixture of phosphate buffer solu-

tion and acetonitril (53:47).

Flow rate : 2.0 mL/minute.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor are not less than 800 and not more than 2, respectively.

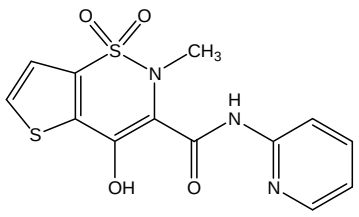
System repeatability: When the test is repeated with 10 μL each of the standard solution under the above operating conditions, the relative deviation of peak area obtained from temazepam is not more than 2.0 %.

phosphate buffer solution—Dissolve 2.7 g of potassium dihydrogen phosphate in 1000 mL of water. Adjust with phosphoric acid to pH of 3.0.

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Tenoxicam



$C_{13}H_{11}N_3O_4S_2$: 337.37

(3*Z*)-3-{Hydroxy[(pyridin-2-yl)amino]methylidene}-2-methyl-2*H*,3*H*,4*H*-1$l^{6},5,2-thieno[2,3-e][1$l^{6},2]thiazine-1,1,4-trione [*59804-37-4*]

Tenoxicam contains not less than 99.0 % and not more than 101.0 % of tenoxicam ($C_{13}H_{11}N_3O_4S_2$), calculated on the anhydrous basis.

Description Tenoxicam is a yellow, crystalline powder.

Tenoxicam is sparingly soluble in dichloromethane, very slightly soluble in ethanol (95), and practically insoluble in water.

Tenoxicam dissoves in a solution of acids or alkalis.

Tenoxicam shows polymorphism.

Identification Determine the infrared spectra with Tenoxicam and Tenoxicam RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears, dissolve Tenoxicam and Tenoxicam RS respectively in the minimum quantity of dichloromethane, evaporate to dryness on a water bath and repeat the test with the residues.

Purity (1) ***Clarity and color of solution***—Dissolve 0.10 g of Tenoxicam in 20 mL of dichloromethane. This solution is clear.

(2) ***Heavy metals***—Proceed with 1.0 g of Tenoxicam according to the Method 2, and perform the test. Prepare the control solution with 2 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—weigh exactly 0.4 g of Tenoxicam, dissolve in a mixture of methanol and ammonia solution (28) (96 : 4) to make exactly 5 mL, and use this solution as the test solution. To 1.0 mL of this solution add a mixture of methanol and ammonia solution (28) (96 : 4) to make exactly 20 mL, to 1.0 mL of this solution add a mixture of methanol and ammonia solution (28) (96:4) to make exactly 20 mL, and use this solution as the standard solution (1). Separately, weigh exactly 20 mg of salicylic acid RS and 20 mg of Tenoxicam RS, dissolve in a mixture of methanol and ammonia solution (28) (96 : 4) to make 5 mL, and use this solution as the standard solution (2). Weigh exactly 20 mg of pyridine-2-amine, dissolve it in a mixture of methanol and ammonia solution (28) (96 : 4) to make exactly 5 mL, add a mixture of methanol and ammonia solution (28) (96 : 4) to 2 mL of this solution to make exactly 50 mL, and use this solution as the standard solution (3). Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot each 10 μL each of the test solution and the standard solutions on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, acetone, methanol and formic acid (70 : 20 : 5 : 5) to a distance of about 15 cm. Air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm). The spot corresponding to pyridine-2-amine in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with standard solution (3), and any spot in the chromatogram obtained with the test solution other than the principal spot and the spot corresponding to pyridine-2-amine, is not more intense than the spot in the chromatogram obtained with standard solution (1).

Water Not more than 0.5 % (1 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

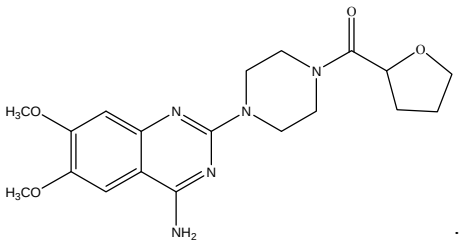
Assay Dissolve 0.25 g of Tenoxicam in 5 mL of formic acid, add 70 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and any necessary correction.

1 mL of 0.1 mol/L perchloric acid
= 33.74 mg of $C_{13}H_{11}N_3O_4S_2$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Terazosin Hydrochloride Hydrate



· HCl · $2H_2O$

$C_{19}H_{25}N_5O_4 \cdot HCl \cdot 2H_2O$: 459.92

6,7-Dimethoxy-2-{4-[(oxolan-2-yl)carbonyl]piperazin-1-yl}quinazolin-4-amine dihydrate hydrochloride [*70024-40-7*]

Terazosin Hydrochloride Hydrate, when dried, contains not less than 98.0 % and not more than 102.0 % of terazosin hydrochloride ($C_{19}H_{25}N_5O_4 \cdot HCl$).

Description Terazosin Hydrochloride Hydrate appears as white to pale yellow crystalline powder.
Terazosin Hydrochloride Hydrate is sparingly soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (95), and practically insoluble in acetone.

Identification (1) Determine the infrared spectra of Terazosin Hydrochloride Hydrate and Terazosin Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

(3) Dissolve 0.1 g of Terazosin Hydrochloride Hydrate in 10 mL of a mixture of methanol and water (9 : 1): the solution responds to the Qualitative Tests for chloride.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Terazosin Hydrochloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Tetrahydro-2-furancarboxylic acid***—Weigh accurately about 0.1 g of Terazosin Hydrochloride Hydrate, transfer to a 50 mL centrifuge tube, add 5.0 mL of acetone and 5.0 mL of the internal standard solution, and shake for about 30 minutes. Centrifuge for about 10 minutes, and filter through a nylon membrane filter (previously washed with acetone) with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the test solution. Separately, weigh accurately an amount of Tetrahydro-2-furancarboxylic Acid RS, and dissolve in acetone so that each mL contains exactly 1.0 mg. Dissolve this solution in acetone so that each mL contains exactly 100 μg, and use this solution as the standard stock solution. Put 5.0 mL of the standard stock solution and 5.0 mL of the internal standard solution into a 50 mL centrifuge tube, and filter through a nylon membrane filter (previously washed with acetone) with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the standard solution. Separately, pipet 2.0 mL of acetic acid (100), and add acetone to make exactly 100 mL. Mix 5.0 mL of this solution with 5.0 mL of acetone, and filter through a nylon membrane filter (previously washed with acetone) with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the blank solution. Perform the test with about 0.2 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of tetrahydro-2-furancarboxylic acid to that of the internal standard (not more than 0.1 %). Confirm that there are no additional peaks when test is performed with about 0.2 μL of the blank solution as directed under Gas Chromatography according to the following conditions.

$$\text{Amount (\%) of tetrahydro-2-furancarboxylic acid} = \frac{C}{W} \times \frac{Q_T}{Q_S}$$

C: Concentration (mg/mL) of tetrahydro-2-furancarboxylic acid in the standard solution

W: Amount (mg) of terazosin hydrochloride in the test solution

Internal standard solution—Weigh accurately about 0.1 g of capric acid, transfer to a 100 mL volumetric flask, dissolve in acetone to make 100 mL, and mix. Mix 10.0 mL of this solution with 2.0 mL of acetic acid (100) in a 100 mL volumetric flask, dilute with acetone to make 100 mL, and mix.

Operating conditions

Detector: A hydrogen flame ionization detector.

Column: A fused-silica column about 0.53 mm in internal diameter and about 10 m in length, coated with polyethylene glycol compound TPA for gas chromatography 1.2 μm in thickness.

Column temperature: A constant temperature of about 170 °C

Injection port temperature: A constant temperature of about 230 °C

Detector temperature: A constant temperature of about 240 °C

Carrier gas: Helium
Flow rate: 9 mL/minute
System suitability

System performance: When the procedure is run with the standard solution under the above operating conditions, the relative retention times of tetrahydro-2-furancarboxylic acid and capric acid are 1.0 and 1.2, respectively, and the resolution between the peaks of tetrahydro-2-furancarboxylic acid and capric acid is not less than 2.3.

System repeatability: When the test is repeated 6 times with the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of tetrahydro-2-furancarboxylic acid is not more than 6.5 %.

(3) ***1-[(Tetrahydro-2-furanyl)piperazine]***—Weigh accurately about 125 mg of Terazosin Hydrochloride Hydrate, dissolve in a mixture of acetonitrile and water (1 : 1) to make exactly 25 mL, and use this solution as the test solution. Separately, weigh accurately an amount of 1-[(Tetrahydro-2-furanyl)piperazine RS, and dissolve in acetonitrile so that each mL contains exactly 1.0 mg. Dissolve this solution in acetonitrile so that each mL contains exactly 5 μg, and use this solution as the standard solution. Use acetonitrile as the blank solution. Separately, dissolve about 2.0 g of 3,5-dinitrobenzoyl chloride in 250 mL of acetonitrile, and use this solution as the derivatization solution. Transfer 5 mL each of the blank solution, standard solution, and test solution to separate 100 mL volumetric flasks, mix with 5.0 mL of phosphate buffer solution, add 10.0 mL of the derivatization solution, allow to stand at room temperature for about 20 minutes, dilute with a mixture of acetonitrile and water (1 : 1) to make 100 mL, and mix. Perform the test with 50 μL each of the derivatized blank solution, derivatized standard solution, and derivatized test solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method. Calculate the content of 1-[(tetrahydro-2-furanyl)piperazine by the following equation: not more than 0.1 %.

$$\text{Content (\%) of 1-[(tetrahydro-2-furanyl)piperazine} = \frac{C}{W} \times \frac{A_T}{A_S} \times 2500$$

C: Concentration (mg/mL) of 1-[(tetrahydro-2-furanyl)piperazine in the standard solution
W: Amount (mg) of terazosin hydrochloride in the test solution

Operating conditions
Detector: An ultraviolet absorption photometer (wavelength: 254 nm)
Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octylsilyl silica gel for liquid chromatography (5 μm in particle diameter).
Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as directed in the following table.
Mobile phase A: Water
Mobile phase B: Acetonitrile

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-35	82	18
35-40	82→10	18→90
40-75	10	90
75-80	10→82	90→18
80-100	82	18

Flow rate: 1.5 mL/minute. Change to 2.0 mL/minute during the period between 40 and 80 minutes.
System suitability

System performance: The retention time of 1-[(tetrahydro-2-furanyl)piperazine is more than 22 minutes, and the number of theoretical plates is not less than 3500.

System repeatability: When the test is repeated 6 times with 50 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of 1-[(tetrahydro-2-furanyl) piperazine is not more than 3.0 %.

Phosphate buffer solution—Weigh accurately about 96.3 g of dipotassium hydrogen phosphate and about 3.85 g of potassium dihydrogen phosphate, transfer to a 500 mL volumetric flask, and dilute with water to make 500 mL. Adjust the pH to 8.0 ± 0.1 with diluted phosphoric acid solution (10 in 100) or sodium hydroxide solution (10 in 100). Transfer 25.0 mL of this solution to a 100 mL volumetric flask, and dilute with water to make 100 mL. Adjust the pH to 8.0 ± 0.1 with diluted phosphoric acid solution (10 in 100) or sodium hydroxide solution (10 in 100).

(4) ***Other related substances***—Proceed with Terazosin Hydrochloride Hydrate in the same manner as the test stock solution in the Assay, and use this solution as the test solution. Separately, dissolve 6.0 g of sodium citrate dihydrate and 4.0 g of citric acid (100) in water to make 1000 mL, and use this solution as diluent (1). Separately, use a mixture of water, acetonitrile, and methanol (60 : 30 : 10) as diluent (2). Separately, weigh accurately Terazosin Related Substance I {1-(4-amino-6,7-dimethoxy-2-quinazolinyl)piperazine, dihydrochloride} RS, dissolve in diluent (1) so that each mL contains exactly 0.5 mg, and use this solution as the related substance standard stock solution (1). Separately, weigh accurately Terazosin Related Substance II {1-(4-hydroxy-6,7-dimethoxy-2-quinazolinyl)-4-[(tetrahydro-2-furanyl)carbonyl]piperazine} RS, dissolve in methanol so that each mL contains exactly 0.5 mg, and use this solution as the related substance standard stock solu-

tion (2). Separately, weigh accurately Terazosin Related Substance III {1,4-bis(4-amino-6,7-dimethoxy-2-quinazolinyl)piperazine, dihydrochloride} RS, dissolve in diluent (2) so that each mL contains exactly 0.1 mg, and use this solution as the related substance standard stock solution (3). Transfer 60 mL of diluent (2) to a 100 mL volumetric flask, add 5.0 mL of the standard stock solution from the Assay, 4.0 mL of the related substance standard stock solution (1), 4.0 mL of the related substance standard stock solution (2), and 20 mL of the related substance standard stock solution (3), and add diluent (2) to make exactly 100 mL. Pipet 10.0 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the area percentage method. Calculate the amount of related substances by the following equation: terazosin related substance I is not more than 0.3 %, terazosin related substance III is not more than 0.4 %, each related substance eluting prior to the peak of terazosin is not more than 0.3 %, any other related substance is not more than 0.1 %, and the total amount of related substances is not more than 0.6 %.

$$\text{Amount (\%) of terazosin related substance I or terazosin related substance III} = C \times \frac{A_T}{A_S} \times 200$$

C: Concentration (mg/mL) of the related substance in the standard solution

A_T: Peak area of the related substance from the test solution

A_S: Peak area of the related substance from the standard solution

$$\text{Amount (\%) each related substance} = C \times \frac{A_i}{A_r} \times 200$$

C: Concentration (mg/mL) of Terazosin Hydrochloride RS in the standard solution

A_T: Peak area of each related substance from the test solution

A_S: Peak area of terazosin from the standard solution

Operating conditions

Detector, column, mobile phase, flow rate, etc.: Proceed as directed in the operating conditions in the Assay.

System suitability

System performance: When the procedure is run with 20 μL fo the standard solution under the above operating conditions, the relative retention times of terazosin related substance I, terazosin, terazosin related substance II, and terazosin related substance III are about 0.2, 1.0, 1.48, and 2.57, respectively. The resolution between the peaks of terazosin and terazosin related substance II is not less than 9.0. The number of theoretical plates of the peak of terazosin is not less than 12000, and the symmetry factor of the peak of terazosin related substance III is not more than 2.5.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviations of the peak areas of terazosin and terazosin related substance III are not more than 2.0 % and not more than 5.0 %, respectively.

Time span of measurement: For 60 minutes after injection of the sample.

Loss on Drying Not more than 9.0 % (1 g, in vacuum, 105 °C, 3 hours).

Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately an amount of Terazosin Hydrochloride Hydrate, equivalent to about 0.1 g of terazosin hydrochloride, dissolve in the mobile phase to make exactly 200 mL, and use this solution as the test stock solution. Pipet 10.0 mL of the test stock solution, and add the mobile phase to make exactly 50 mL. Pipet 10.0 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the test solution. Separately, weigh accurately an amount of Terazosin Hydrochloride RS, dissolve in the mobile phase so that each mL contains 0.5 mg of terazosin hydrochloride, and use this solution as the standard stock solution. Pipet 1.0 mL of the standard stock solution, and add the mobile phase to make exactly 50 mL. Pipet 10.0 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of terazosin hydrochloride in each solution.

$$\text{Amount (mg) of terazosin hydrochloride } (C_{19}H_{25}N_5O_4 \cdot HCl) = C \times \frac{A_T}{A_S} \times 10000$$

C: Concentration (mg/mL) of Terazosin Hydrochloride RS in the standard solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30 °C

Mobile phase: A mixture of pH 3.2 citrate buffer solution and acetonitrile (1685 : 315)

Flow rate: 1.0 mL/minute

Systemsuitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor are not less than 12000 and 0.9 to 1.3, respectively.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area is not more than 0.9 %.

pH 3.2 Citrate buffer—Dissolve 12.0 g of sodium citrate dihydrate and 28.5 g of citric acid (100) in 1950 mL of water. Adjust the pH to 3.2 ± 0.1 with citric acid (100) or sodium citrate, and add water to make 2000 mL.

Containers and Storage ***Containers***—Tight containers.

Storage—At a temperature between 20 and 25 °C.

Terazosin Hydrochloride Tablets

Terazosin Hydrochloride Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of terazosin ($C_{19}H_{25}N_5O_4$: 387.43).

Method of Preparation Prepare as directed under Tablets, with Terazosin Hydrochloride.

Identification (1) Transfer an amount of powdered Terazosin Hydrochloride Tablets, equivalent to 10 mg of terazosin hydrochloride according to the labeled amount, to a 100 mL volumetric flask, dilute with 0.1 mol/L hydrochloric acid to 50 % of the volume of the flask, sonicate for 10 minutes, cool to room temperature, and add 0.1 mol/L hydrochloric acid to volume. To 5 mL of this solution add 0.1 mol/L hydrochloric acid to make 100 mL, mix, filter 20 mL of this solution through a membrane filter with a pore size of 0.45 μm, discard the first 5 mL of the filtrate, and use the subsequent filtrate as the test solution. Separately, dissolve Terazosin Hydrochloride RS in 0.1 mol/L hydrochloric acid so that each mL contains 0.005 mg/mL, sonicate for 10 minutes to dissolve completely, filter through a nylon filter with a pore size of 0.45 μm, discard the first 5 mL of the filtrate, and use the subsequent filtrate as the standard solution. Determine the absorption spectra of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit maxima and minima at the same wavelengths.

(2) The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

Purity ***Related substances***—Weigh accurately and powder not less than 20 Terazosin Hydrochloride Tablets. Weigh accurately a portion of the powder, equivalent to about 15 mg of terazosin hydrochloride, transfer to a 50 mL volumetric flask, dilute with 25 mL of the mobile phase, sonicate for not less than 10 minutes, and shake for not less than 20 minutes. To this solution add the mobile phase to make 50 mL. Filter this solution through a nylon or Teflon filter with a pore size of 0.45 μm, discard the first 5 mL of the filtrate, and use the subsequent filtrate as the test solution. Separately, dissolve Terazosin Hydrochloride RS in the mobile phase so that each mL contains 0.003 mg, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, determine each peak area of each solution by the automatic integration method, and calculate the amount of related substances in the test solution: the amounts of terazosin related substance I {N-(4-amino-6,7-dimethoxy-2-quinazolinyl)piperazine}, terazosin related substance II {2-chloro.4-amino-6,7-dimethoxy-2-quinazoline}, and terazosin related substance III {*N,N*-bis-(4-amino-6,7-dimethoxy-2-quinazolinyl)piperazine}, having the relative retention times of about 0.52, about 1.37, and about 3.85 with respect to terazosin hydrochloride, respectively, are not more than 0.4 %, respectively. Any other related substance is not more than 0.2 %, and the total amount of related substances is not more than 1.2 %. The relative response factors with respect to the peak areas of terazosin related substance I and terazosin related substance II are 1.1 and 1.2, respectively.

$$\text{Amount (\%) of related substances} = \frac{A_T}{A_S} \times \frac{C_S}{C_T} \times \frac{1}{F} \times \frac{387.43}{423.89} \times 100$$

C_S: Concentration (mg/mL) of terazosin hydrochloride in the standard solution

C_T: Concentration (mg/mL) of terazosin in the test solution

A_T: Area of each related substance from the test solution

A_S: Area of terazosin hydrochloride from the standard solution

F: Relative response factor

387.43: Molecular mass of terazosin

423.89: Molecular mass of terazosin hydrochloride

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 246 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanizec silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of the buffer solution and acetonitrile (19 : 6)

Flow rate: 1 mL/minute
System suitability
System performance: Dissolve Terazosin Hydrochloride RS in the mobile phase so that each mL contains 0.15 μg, and use this solution as the system suitability solution. When the procedure is run with 10 μL each of the standard solution and system suitability solution under the above operating conditions, the capacity factor of the peak of terazosin from the standard solution s not less than 1.0 with the symmetry factor being not more than 2.0, and the signal-to-noise ratio of the peak of terazosin from the system suitability solution is not less than 10.
System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of terazosin is not more than 2.0 %.
Time span of measurement: About 4.5 times as long as the retention time of terazosin, beginning after the solvent peak.

Buffer solution—Dissolve 4.1 g of potassium dihydrogen phosphate and 1.1 g of sodium 1-heptanesulfonate monohydrate in 950 mL of water, adjust the pH to 3.0 ± 0.10 with phosphoric acid, dilute with water to make 1000 mL, filter through a nylon filter with a pore size of 0.45 μm and use the filtrate as the buffer solution.

Dissolution Test Perform the test with 1 tablet of Terazosin Hydrochloride Tablets at 50 revolutions per minute according to Method 2 under Dissolution Test, using 900 mL of water as the dissolution solution. Take 20 mL of the dissolved solution 30 minutes after the start of the test, and filter through a membrane filter with a pore size of 0.45 μm. Discard the first 10 mL of the filtrate, and to the subsequent filtrate add water so that each mL contains about 5 μg of terazosin ($C_{19}H_{25}N_5O_4$), and use this solution as the test solution. Separately, dissolve Terazosin Hydrochloride RS in water so that each mL contains 0.1 mg of terazosin hydrochloride. To 5.0 mL of this solution add water to make 100.0 mL, and use this solution as the standard solution. Detemine the absorbances at 245 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry. The dissolution rate of Terazosin Hydrochloride Tablets in 30 minutes is not less than 75 %.

$$\text{Dissolution rate (\%) of terazosin } (C_{19}H_{25}N_5O_4) = \frac{A_T}{A_S} \times \frac{C_S}{L} \times \frac{387.43}{423.89} \times V \times 100$$

A_T: Absorbance of the test solution
A_S: Absorbance of the standard solution
C_S: Concentration (mg/mL) of terazosin hydrochloride in the standard solution
L: Labeled amount (mg/tablet) of terazosin in 1 tablet
387.43: Molecular mass of terazosin
423.89: Molecular mass of terazosin hydrochloride
V: Volume of the dissolution solution, 900 mL

Uniformity of Dosage Units It meets the requirement when the content uniformity test is performed with 1 tablet of Terazosin Hydrochloride Tablets as directed in the Assay.

Assay Weigh accurately and powder not less than 20 Terazosin Hydrochloride Tablets. Weigh accurately a portion of the powder, equivalent to about 10 mg of terazosin hydrochloride ($C_{19}H_{25}N_5O_4 \cdot HCl$), transfer to a 200 mL volumetric flask, add 100 mL of the diluent, sonicate for not less than 10 minutes, and shake for not less than 10 minutes. Repeat this procedure until the sample is uniformly dispersed. Cool at an ordinary temperature, and add the diluent to make 200 mL. Filter through a membrane filter with a pore size of 0.45 μm, discard the first 5 mL of the filtrate, and use the subsequent filtrate as the test solution. Separately, dissolve Terazosin Hydrochloride RS in the diluent so that each mL contains 0.55 mg of terazosin hydrochloride, sonicate for 5 minutes to dissolve completely, and use this solution as the standard stock solution. To the standard stock solution add the diluent so that each mL contains 0.055 mg of terazosin hydrochloride. Filter through a membrane filter with a pore size of 0.45 μm, discard the first 5 mL of the filtrate, and use the subsequent filtrate as the standard solution. Perform the test with 25 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of terazosin hydrochloride.

$$\text{Amount (\%) of terazosin } (C_{19}H_{25}N_5O_4) = \frac{A_T}{A_S} \times \frac{C_S}{C_T} \times \frac{387.43}{423.89} \times 100$$

C_S: Concentration (mg/mL) of terazosin hydrochloride in the standard solution
C_T: Concentration (mg/mL) of terazosin in the test solution
387.43: Molecular mass of terazosin
423.89: Molecular mass of terazosin hydrochloride

Diluent—Mix 1000 mL of methanol with 0.85 mL of hydrochloric acid to make a 0.01 mol/L hydrochloric acid-methanol solution. Use a mixture of 0.01 mol/L hydrochloric acid-methanol solution and water (2 : 3) as the diluent.

Operating conditions
Detector: An ultraviolet absorption photometer (wavelength: 254 nm)
Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: To a mixture of acetonitrile and water (7 : 3) add acetic acid (100) so that each L contains 10.00 mL, degas, filter through a nylon filter with a pore size of 0.45 μm, to the filtrate add 0.20 mL of diethylamine, and mix.

Flow rate: 1 mL/minute

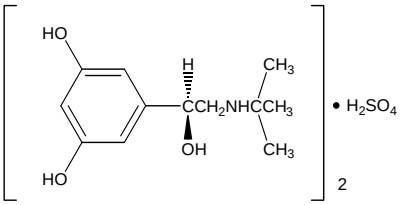
System suitability

System performance: Put 50 mg of Naproxen RS into a 100 mL volumetric flask, add 25 mL of acetonitrile, sonicate to dissolve, add water to make 100 mL, and use this solution as the naproxen standard solution. To the naproxen standard solution and the standard stock solution from the Assay add the diluent so that each mL contains 0.05 mg of naproxen and 0.055 mg of terazosin, respectively, filter through a membrane filter with a pore size of 0.45 μm, discard the first 5 mL of the filtrate, and use the subsequent filtrate as the system suitability solution. When the procedure is run with 25 μL of the system suitability solution under the above operating conditions, the resolution between the peaks of naproxen and terazosin is not less than 2.0, and the symmetry factor of the peak of terazosin is not more than 1.8.

System repeatability: When the test is repeated 6 tmies with 25 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of terazosin is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Terbutaline Sulfate



and enantiomer

$(C_{12}H_{19}NO_3)_2H_2SO_4$: 548.65

5-[2-(*tert*-Butylamino)-1-hydroxyethyl]benzene-1,3-diol; sulfuric acid, [*23031-32-5*]

Terbutaline Sulfate contains not less than 98.5 % and not more than 101.0 % of terbutaline sulfate $[(C_{12}H_{19}NO_3)_2H_2SO_4]$, calculated on the anhydrous basis.

Description Terbutaline Sulfate appears as white to brownish white crystals or crystalline powder, is odorless or has a faint odor of acetic acid.

Terbutaline Sulfate is freely soluble in water and practically insoluble in acetonitrile, in acetic acid (100), in ethanol (95), in chloroform or in ether.

Terbutaline Sulfate is gradually colored by light and by air.

Melting point—About 255 °C (with decomposition).

Identification (1) Dissolve 1 mg of Terbutaline Sulfate in 1 mL of water and add 5 mL of Tris buffer solution, pH 9.5, 0.5 mL of 4-aminoantipyrine solution (1 in 50) and 2 drops of potassium hexacyanoferrate (III) solution (2 in 25): a red-purple color is observed.

(2) Determine the absorption spectra of solutions of Terbutaline Sulfate and Terbutaline Sulfate RS in 0.01 mol/L hydrochloric acid TS (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths. The maximum may be biphasic.

(3) A solution of Terbutaline Sulfate (1 in 50) responds to the Qualitative Tests for sulfate.

pH Dissolve 0.10 g of Terbutaline Sulfate in 10 mL of water: the pH of this solution is between 4.0 and 4.8.

Purity (1) ***Clarity and color of solution***—Dissolve 0.10 g of Terbutaline Sulfate in 10 mL of water: the solution is clear and colorless or pale yellow.

(2) ***Chloride***—Perform the test with 2.0 g of Terbutaline Sulfate. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.004 %).

(3) ***Heavy metals***—Proceed with 2.0 g of Terbutaline Sulfate as directed under Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(4) ***Arsenic***—Prepare the test solution with 1.0 g of Terbutaline Sulfate according to method 3 and perform the test (not more than 2 ppm).

(5) ***Related substances***—Weigh accurately 50 mg of Terbutaline Sulfate, add the mobile phase to make 50 mL, and use this solution as the test solution. Separately, weigh accurately 30 mg of Terbutaline Sulfate RS, dissolve in the mobile phase to make 100 mL, pipet 1.0 mL of this solution, add the mobile phase to make 100 mL so that each mL contains about 3 μg of terbutaline sulfate, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas of each solution by the automatic integration method: the total amount of related substances is not more than 1.0 %.

$$\text{Amount (\%) of related substances} = 5000 \times \frac{C}{W} \times \frac{A_T}{A_S}$$

C: Concentration (mg/mL) of terbutaline sulfate in the standard solution

W: Amount (mg) of sample taken

A_T: Peak area of each related substance obtained from the test solution

A_S: Peak area of terbutaline obtained from the standard solution

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 276 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Dissolve 3.15 g of ammonium formate in 900 mL of water, adjust the pH to 3.0 with formic acid, add 5.49 g of sodium 1-hexanesulfonate, and add water to make 1000 mL. To 770 mL of this solution add 230 mL of methanol.

Flow rate: 1 mL/minute

System suitability

System performance: Weigh accurately 25 mg of Terbutaline Sulfate RS and 10 mg of Terbutaline Related Substance I {3,5-dihydroxy-ω-t-butylaminoacetophenone sulfate} RS, dissolve in the mobile phase to make 25 mL so that each mL contains 1.0 mg and 0.4 mg, respectively, and use this solution as the system suitability solution. When the procedure is run with 20 μL of this solution under the above operating conditions, the relative retention time of terbutaline related substance I with respect to the retention time of terbutaline is 0.9, with the resolution between these peaks being not less than 2.0, and the number of theoretical plates and symmetry factor of the peak of terbutaline are not less than 1500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 5 times with 20 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the peak area of terbutaline is not more than 2.0 %.

(6) ***3,5-Dihydroxy-ω-tert-butylaminoacetophenone sulfate***—Dissolve 0.50 g of Terbutaline Sulfate in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry: the absorbance at 330 nm is not more than 0.47.

(7) ***Acetic acid***—Dissolve about 0.50 g of Terbutaline Sulfate in a solution of phosphoric acid (59 in 1000) to make exactly 10 mL and use this solution as the test solution. Separately, dissolve 1.50 g of acetic acid (100) in a solution of phosphoric acid (59 in 1000) to make exactly 100 mL. Dilute 2 mL of this solution, accurately measured, with a solution of phosphoric acid (59 in 1000) to make exactly 200 mL and use this solution as the standard solution. Perform the test with 2 μL each of the test solution and the standard solution as directed under Gas Chromatography according to the following operating conditions. Measure the peak areas, A_T and A_S, of acetic acid for the test solution and the standard solution, respectively: A_T is not larger than A_S.

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A column, about 3 mm in internal diameter and about 1 m in length, packed with terephthalic acid for gas chromatography (180 μm to 250 μm in particle diameter), coated with macrogol 6000 at the ratio of 10 %.

Column temperature: A constant temperature of about 120 °C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of acetic acid is about 5 minutes.

System suitability

System performance: Dissolve 50 mg each of acetic acid (100) and propionic acid in 100 mL of a solution of phosphoric acid (59 in 1000). When the procedure is run with 2 μL of this solution under the above operating conditions, acetic acid and propionic acid are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 2 μL each of standard solution under the above operating conditions, the relative standard deviation of the peak area of acetic acid is not more than 3.0 %.

Water Not more than 0.5 % (1 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.2 % (1 g).

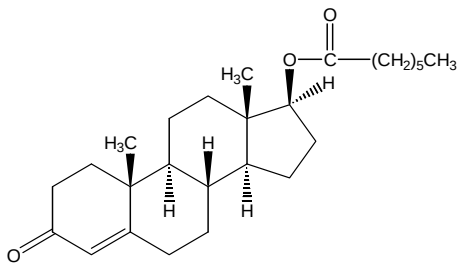
Assay Weigh accurately about 0.5 g of Terbutaline Sulfate, dissolve in 50 mL of a mixture of acetonitrile and acetic acid (100) (1 : 1) by stirring and warming. After cooling, titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry, substituting a saturated solution of potassium chloride in methanol for the internal solution).

Each mL of 0.1 mol/L perchloric acid VS
= 54.87 mg of $(C_{12}H_{19}NO_3)_2 \cdot H_2SO_4$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Testosterone Enanthate



$C_{26}H_{40}O_3$: 400.59

[(8*R*,9*S*,10*R*,13*S*,14*S*,17*S*)-10,13-Dimethyl-3-oxo-1,2,6,7,8,9,11,12,14,15,16,17-dodecahydrocyclopenta[a]phenanthren-17-yl]heptanoate [*315-37-7*]

Testosterone Enanthate, when dried, contains not less than 95.0 % and not more than 105.0 % of testosterone enanthate ($C_{26}H_{40}O_3$).

Description Testosterone Enanthate appears as white to pale yellow crystals, crystalline powder or a pale yellow-brown, viscous liquid and is odorless or has a slight characteristic odor.
Testosterone Enanthate is very soluble in ethanol (95), in 1,4-dioxane or in ether and practically insoluble in water.

Melting point—About 36 °C.

Identification Heat 25 mg of Testosterone Enanthate with 2 mL of a solution of potassium hydroxide in methanol (1 in 100) under a reflux condenser in a water-bath for 1 hour. After cooling, add 10 mL of water, suction filter the precipitate, wash the residue with water until the last washing is neutral and dry the residue in a desiccator (in vacuum, P_2O_5) for 4 hours: it melts between 151 °C and 157 °C.

Specific Optical Rotation $[\alpha]_D^{20}$: +81 ~ +86° (0.1 g after drying, ethanol (99.5), 10 mL, 100 mm).

Purity (1) ***Acid***—Dissolve 0.5 g of Testosterone Enanthate in 10 mL of ethanol which has previously been rendered neutral to bromothymol blue TS and add 2 drops of bromothymol blue TS and 0.50 mL of 0.01 mol/L sodium hydroxide VS: the color of the solution is pale blue.

(2) ***Free heptanoic acid***—Weigh accurately 0.5 g of Testosterone Enanthate, dissolve in 10 mL of ethanol, previously neutralized to pale blue with 2 to 3 drops of bromothymol blue TS, and titrate with 0.01 mol/L sodium hydroxide VS: not less than 0.6 mL of sodium hydroxide VS is consumed (not more than 0.16 % of heptanoic acid).

(3) ***Related substances***—Weigh accurately 0.1 g of Testosterone Enanthate, dissolve in methanol to make exactly 10 mL so that each mL contains about 10 mg, and use this solution as the test solution. Separately, weigh accurately a suitable amount of Testosterone Enanthate RS, dissolve in methanol so that each mL contains about 0.01 mg, 0.05 mg, 0.1 mg, and 0.2 mg, and use these solutions as the standard solutions (1), (2), (3), and (4), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 20 μL each of the test solution and standard solutions (1), (2), (3), and (4) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and ethyl acetate (2 : 1) to a distance of about 3/4 of the length of the plate, and air-dry the plate. Spray evenly a solution prepared by dissolving 20 g of *p*-toluenesulfonic acid monohydrate in 100 mL of alcohol on the plate, dry at 110 °C for 15 minutes, and examine under ultraviolet light (main wavelength: 366 nm): no spot obtained from the test solution is larger or more intense than the principal spot from the standard solution (3) (not more than 1.0 %). The total amount of the spots other than the principal spot from the test solution is not more than 2.0 %.

Loss on Drying Not more than 0.5 % (0.5 g, in vacuum, P_2O_5, 4 hours).

Residue on Ignition Not more than 0.1 % (0.5 g).

Assay Weigh accurately about 0.1 g of Testosterone Enanthate, previously dried and dissolve in ethanol (95) to make exactly 100 mL. Pipet 10.0 mL of this solution and dilute with ethanol (95) to make exactly 100 mL. Pipet 10.0 mL of this solution and dilute with ethanol (95) to make exactly 100 mL. Perform the test as directed under Ultraviolet-visible Spectrophotometry with this solution. Read the absorbance, *A*, of this solution at the wavelength of a maximum absorption at about 241 nm.

$$\text{Amount (mg) of testosterone enanthate } (C_{26}H_{40}O_3) = \frac{A}{426} \times 100000$$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and not exceeding 30 °C.

Testosterone Enanthate Injection

Testosterone Enanthate Injection is an oily solution for injection. Testosterone Enanthate Injection contains not less than 90.0 % and not more than 110.0 % of the labeled amount of testosterone enanthate ($C_{26}H_{40}O_3$: 400.59).

Method of Preparation Prepare as directed under Injections, with Testosterone Enanthate.

Descriptlon Testosterone Enanthate Injection is a clear, colorless or pale yellow oily liquid.

Identification Measure a volume of Testosterone Enanthate Injection, equivalent to 50 mg of Testosterone Enanthate according to the labeled amount, add 8 mL of petroleum ether and extract with three 10 mL volumes of diluted acetic acid (7 in 10). Combine the extracts, wash with 10 mL of petroleum ether, add 0.5 mL of diluted sulfuric acid (7 in 10) to 0.1 mL of the extract and heat in a water-bath for 5 minutes. After cooling, add 0.5 mL of iron (III) chloride-acetic acid TS: the color of the solution is blue.

Sterility Test It meets the requirement.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay Measure accurately a volume of Testosterone Enanthate Injection, equivalent to about 50 mg of testosterone enanthate ($C_{26}H_{40}O_3$) according to the labeled amount, and dissolve in chloroform to make exactly 100 mL. Pipet 15.0 mL of this solution, add 1.0 mL of internal standard solution and chloroform to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately about 50 mg of Testosterone Enanthate RS, previously dried in a desiccator (in vacuum, P_2O_5) for 4 hours, proceed in the same manner as for the test solution and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under the Liquid chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of testosterone enanthate to that of internal standard, for each solution.

$$\text{Amount (mg) of testosterone enanthate } (C_{26}H_{40}O_3) = \text{Amount (mg) of Testosterone Enanthate RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of phenanthrene in methanol (1 in 2000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Column temperature: An ordinary temperature.

Mobile phase: A mixture of acetonitrile and water (85 : 15)

Flow rate: Adjust the flow rate so that the retention time of Testosterone Enanthate is about 10 minutes.

System suitablilty

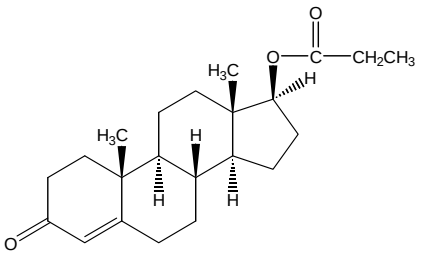
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions: internal standard and testosterone enanthate are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 20 μL each of standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of testosterone enanthate to that of the internal standard is not more than 2.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Storage—Light-resistant.

Testosterone Propionate



$C_{22}H_{32}O_3$: 344.49

[(8*R*,9*S*,10*R*,13*S*,14*S*,17*S*)-10,13-Dimethyl-3-oxo-1,2,6,7,8,9,11,12,14,15,16,17-dodecahydrocyclopenta[a]phenanthren-17-yl]propanoate [*57-85-2*]

Testosterone Propionate, when dried, contains not less than 97.0 % and not more than 103.0 % of testosterone propionate ($C_{22}H_{32}O_3$).

Description Testosterone Propionate appears as white to pale yellow crystals or crystalline powder and is odorless.

Testosterone Propionate is freely soluble in methanol or in ethanol (95), and practically insoluble in water.

Identification (1) Determine the absorption spectra of solutions of Testosterone Propionate and Testosterone Propionate RS in ethanol (95) (1 in 100000), as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Testosterone Propionate and Testosterone Propionate RS, previously dried, as directed under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +83 ~ +90° (0.1 g after drying, ethanol (95), 10 mL, 100 mm).

Melting Point 118 ~ 123 ºC.

Purity ***Related substances***—Dissolve 40.0 mg of Testosterone Propionate in 2 mL of ethanol (95) and use this solution as the test solution. Pipet 1.0 mL of this solution, add ethanol (95) to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution

on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and diethylamine (19 : 1) to a distance of about 15 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (0.5 g, in vacuum, P_2O_5, 4 hours).

Residue on Ignition Not more than 0.1 % (0.5 g).

Assay Weigh accurately each about 10 mg of Testosterone Propionate and Testosterone Propionate RS, previously dried, and dissolve in methanol to make exactly 100 mL, respectively. To exactly 5.0 mL of these solutions, add exactly 5 mL of the internal standard solution and methanol to make exactly 20 mL, and use these solutions as the test solution and the standard solution. Perform the test with 5 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, determine the ratios Q_T and Q_S, of the peak area of Tetosterone Propionate to that of the internal standard.

$$\text{Amount (mg) of testosterone propionate } (C_{22}H_{32}O_3) = \text{Amount (mg) of Testosterone Propionate RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of progesterone in methanol (9 in 100000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 241 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35 ºC.

Mobile phase: A mixture of acetonitrile and water (7 : 3).

Flow rate: Adjust the flow rate so that the retention time of Testosterone Propionate is about 10 minutes.

System suitability

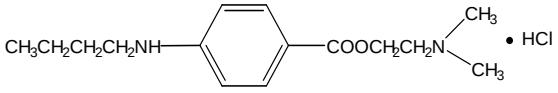
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the internal standard and testosterone propionate are eluted in this order, with the resolution between their peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of peak area of testosterone propionate to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Tetracaine Hydrochloride



$C_{15}H_{24}N_2O_2 \cdot HCl$: 300.82

2-(Dimethylamino)ethyl 4-(butylamino)benzoate hydrochloride [*136-47-0*]

Tetracaine Hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of tetracaine hydrochloride ($C_{15}H_{24}N_2O_2 \cdot HCl$).

Description Tetracaine Hydrochloride appears as white crystals or crystalline powder, is odorless and has a slightly bitter taste followed by a sense of numbness on the tongue.

Tetracaine Hydrochloride is very soluble in formic acid, freely soluble in water, soluble in ethanol (95), sparingly soluble in ethanol (99.5), slightly soluble in acetic anhydride and practically insoluble in ether.

A solution of Tetracaine Hydrochloride (1 in 10) is neutral.

Melting point—About 148 °C.

Identification (1) Dissolve 0.5 g of Tetracaine Hydrochloride in 50 mL of water, add 5 mL of ammonia TS, shake, allow to stand in a cold place and collect the precipitate. Wash with water until the washings is neutral and dry in a desiccator (silica gel) for 24 hours: it melts between 42 °C and 44 °C.

(2) Dissolve 0.1 g of Tetracaine Hydrochloride in 8 mL of water and add 3 mL of ammonium thiocyanate TS: a crystalline precipitate is produced. Filter and collect the precipitate, recrystallize from water and dry at 80 °C for 2 hours: it melts between 130 °C and 132 °C.

(3) Determine the absorption spectra of solutions of Tetracaine Hydrochloride and Tetracaine Hydrochloride RS in ethanol (99.5) (1 in 200000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) A solution of Tetracaine Hydrochloride (1 in 10) respond to the Qualitative Tests for chloride.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Tetracaine Hydrochloride according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Dissolve 0.5 g of

Tetracaine Hydrochloride in water to make 10 mL so that each mL contains 50 mg, and use this solution as the test solution. Separately, dissolve 20 mg of 4-(butylamino)benzoic acid in methanol to make exactly 100 mL so that each mL contains about 0.2 mg, and use this solution as the standard solution. Spot 5 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, and isopropylamine (98 : 7 : 2) to a distance of about 3/4 of the length of the plate, and dry in warm air. Examine under ultraviolet light (254 nm): any spot other than the principal spot obtained from the test solution is not larger or more intense than the principal spot from the standard solution (not more than 0.4 %). The total amount of the spots other than the principal spot from the test solution is not more than 0.8 %.

Loss on Drying Not more than 1.0 % (1 g, 105 °C, 4 hours).

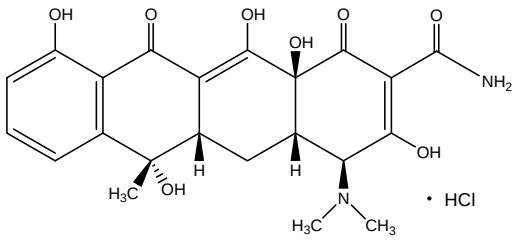
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.5 g of Tetracaine Hydrochloride, previously dried, dissolve in 2 mL of formic acid, add 80 mL of acetic anhydride, allow to stand at 30 °C in a water-bath for 15 minutes, cool and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 30.082 mg of $C_{15}H_{24}N_2O_2$.HCl

Containers and Storage ***Containers***—Tight containers.

Tetracycline Hydrochloride



$C_{22}H_{24}N_2O_8 \cdot HCl$: 480.90

(4*S*,4a*S*,5a*S*,6*S*,12a*S*)-4-(Dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide [*64-75-5*]

Tetracycline Hydrochloride is the hydrochloride of a tetracycline substance having antibacterial activity produced by the growth of *Streptomyces aureofaciens*

Tetracycline Hydrochloride contains not less than 950 μg (potency) and not more than 1010 μg (potency) per mg of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$), calculated on the dried basis.

Description Tetracycline Hydrochloride appears as yellow to pale brownish yellow crystalline powder.
Tetracycline Hydrochloride is freely soluble in water, and sparingly soluble in ethanol (95).

Identification (1) Determine the absorption spectra of solutions of Tetracycline Hydrochloride and Tetracycline Hydrochloride RS (1 in 62500) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Tetracycline Hydrochloride and Tetracycline Hydrochloride RS as directed in the potassium chloride disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Tetracycline Hydrochloride (1 in 100) responds to the Qualitative Tests (2) for chloride.

pH The pH of a solution obtained by dissolving 0.1 g of Tetracycline Hydrochloride in 10 mL of water is between 1.8 and 2.8.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Tetracycline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Arsenic***—Prepare the test solution with 1.0 g of Tetracycline Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

(3) ***Related substances***—Weigh accurately about 25 mg of Tetracycline Hydrochloride, dissolve in exactly 50 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the test solution. Pipet 3 mL of the test solution, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas of each solution by the automatic integration method: the area of each peak other than tetracycline from the test solution is not larger than the peak area of tetracycline from the standard solution. The total area of the peaks other than tetracycline from the test solution is not larger than 3 times the peak area of tetracycline from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (0.01 μm in a pore size).

Column temperature: 60 °C

Mobile phase: Dissolve 3.5 g of dipotassium hydrogen phosphate, 2.0 g of tetrabutylammonium hydrogen sulfate, and 0.4 g of disodium dihydrogen ethylenediaminetetraacetate dihydrate in 300 mL of water, and adjust the pH to 9.0 with sodium hydroxide TS. To this solution add 90.0 g of *t*-butyl alcohol, and add water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of tetracycline is about 5 minutes.

System suitability

Test for required detectability: Pipet 3 mL of the standard solution, and add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Confirm that the peak area of tetracycline obtained from 20 μL of this solution is equivalent to 1 to 5 % of that from the standard solution.

System performance: Dissolve about 0.05 g of Tetracycline Hydrochloride RS in 25 mL of water. Heat 5 mL of this solution in a water bath for 60 minutes, and add water to make 25 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, the retention time of 4-epitetracycline is about 3 minutes, and 4-epitetracycline and tetracycline are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tetracycline is not more than 1.0 %.

Time span of measurement: About 7 times as long as the retention time of tetracycline, beginning after the solvent peak.

Loss on Drying Not more than 2.0 % (1.0 g, in vacuum, 60 °C, 3 hours).

Residue on Ignition Not more than 0.3 % (1.0 g).

Sterility Test It meets the requirement, when Tetracycline Hydrochloride is used in a sterile preparation.

Bacterial Endotoxins Less than 0.5 EU/mg (potency) of tetracycline hydrochloride, when Tetracycline Hydrochloride is used in a sterile preparation.

Histamine It meets the requirement, when Tetracycline Hydrochloride is used in a sterile preparation. Weigh an appropriate amount of Tetracycline Hydrochloride, dissolve in Isotonic Sodium Chloride Injection to make a solution so that each mL contains 5.0 mg (potency), and use the solution as the test solution. Use 0.5 mL of this solution for the test.

Assay Weigh accurately about 50 mg (potency) each of Tetracycline Hydrochloride and Tetracycline Hydrochloride RS, dissolve each in the mobile phase A to make exactly 100 mL, and use these solutions as the test solution and standard solution, respectively. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of tetracycline hydrochloride in the test solution and standard solution.

$$\text{Amount [μg (potency)] of tetracycline hydrochloride } (C_{22}H_{24}N_2O_8 \cdot HCl) = \text{Amount [μg (potency)] of Tetracycline Hydrochloride RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octylsilyl silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Adjust the pH of a mixture of the mobile phase A and mobile phase B (950 : 50) to 7.6 to 7.7 with 3 mol/L ammonium hydroxide and phosphoric acid.

Mobile phase A: A mixture of 0.1 mol/L ammonium oxalate and dimethylformamide (680 : 270)

Mobile phase B: 0.2 mol/L ammonium monohydrogen phosphate

Flow rate: 2.0 mL/minute

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Tetracycline Hydrochloride Capsules

Tetracycline Hydrochloride Capsules contains not less than 90.0 % and not more than 120.0 % of the labeled amount of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$: 480.90).

Method of Preparation Prepare as directed under Capsules, with Tetracycline Hydrochloride.

Identification (1) To an amount of the contents of Tetracycline Hydrochloride Capsules, equivalent to 2 to 3 mg of tetracycline, add 2 mL of sulfuric acid: a red-purple color develops.

(2) The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

Purity ***4-Epianhydrotetracycline***—Weigh accurately the mass of the contents of not less than 20 Tetracycline Hydrochloride Capsules. Weigh accurately an amount of the contents, equivalent to about 50 mg (potency) according to the labeled potency, dissolve in the

mobile phase A to make exactly 100 mL, and use this solution as the test solution. Separately, weigh accurately about 10 mg (potency) of 4-Epianhydrotetracycline Hydrochloride RS, dissolve in mobile phase A to make exactly 1000 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of 4-epianhydrotetracycline in each solution (not more than 3.0 %).

$$\text{Amount (\%) of 4-epianhydrotetracycline} = 10 \times \frac{C_S}{T} \times \frac{A_i}{A_S}$$

C_S: Concentration [μg (potency)/mL] of 4-epianhydrotetracycline hydrochloride in the standard solution

T: Amount [mg (potency)] of tetracycline hydrochloride in the test solution

A_i: Peak area of 4-epianhydrotetracycline from the test solution

A_S: Peak area of 4-epianhydrotetracycline from the standard solution

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Loss on Drying Not more than 4.0 % (0.1 g, 0.7 kPa, 60 °C, 3 hours).

Dissolution Test Perform the test with 1 capsule of Tetracycline Hydrochloride Capsules at 75 revolutions per minute according to Method 2, using 900 mL of water as the dissolution solution. Maintain a distance of 45 ± 5 mm between the stirring blade and the inside bottom of the vessel during the procedure. Take the dissolved solution 60 minutes after the start of the test, or 90 minutes after the start of the test for 0.5 g capsules, and filter. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution solution to make exactly V' mL, and use this solution s the test solution. Separately, weigh accurately a suitable amount of Tetracycline Hydrochloride RS, dissolve in the dissolution solution to make the same concentration as the test solution, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 276 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry, using the dissolution solution as the blank. The dissolution rate of Tetracycline Hydrochloride Capsules in 60 minutes is not less than 80 % (Q). For 0.5 g capsules, the dissolution rate in 90 minutes is not less than 80 % (Q).

$$\text{Dissolution rate (\%) with respect to the labeled amount of tetracycline hydrochloride } (C_{22}H_{24}N_2O_8 \cdot HCl) = C_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 90000$$

C_S: Concentration [mg (potency)/mL] of the standard solution

C: Labeled amount [mg (potency)] of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$) in 1 capsule

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 20 Tetracycline Hydrochloride Capsules. Weigh accurately an amount of the contents, equivalent to about 50 mg (potency) according to the labeled potency, dissolve in mobile phase A to make exactly 100 mL, and use this solution as the test solution. Separately, weigh accurately about 50 mg (potency) of Tetracycline Hydrochloride RS, dissolve in mobile phase A to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of tetracycline hydrochloride in each solution.

$$\text{Amount [μg (potency)] of tetracycline hydrochloride } (C_{22}H_{24}N_2O_8 \cdot HCl) = \text{Amount [μg (potency)] of Tetracycline Hydrochloride RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 250 mm in length, packed with octylsilyl silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase: Adjust the pH of a mixture of the mobile phase A and mobile phase B (950 : 50) to 7.6 to 7.7 with 3 mol/L ammonium hydroxide and phosphoric acid.

Mobile phase A: A mixture of 0.1 mol/L ammonium oxalate TS and dimethylformamide (680 : 270)

Mobile phase B: 0.2 mol/L ammonium monohydrogen phosphate

Flow rate: 2.0 mL/minute

Containers and Storage ***Containers***—Tight containers.

Tetracycline Hydrochloride Ointment

Tetracycline Hydrochloride Ointment contains not less than 90.0 % and not more than 120.0 % of the labeled

amount of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$: 480.90).

Method of Preparation Prepare as directed under Ointments, with Tetracycline Hydrochloride.

Identification (1) To 3.5 g of Tetracycline Hydrochloride Ointment add 20 mL of water, warm in a water bath, filter, take a portion of the filtrate, evaporate to dryness in a water bath, and to the residue add a few drops of sulfuric acid: a reddish purple color develops at first.

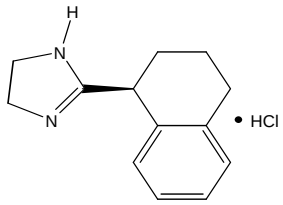
(2) To a portion of the filtrate from (1) add 2 drops of a mixture of iron (III) chloride TS and ethanol (95) (1 : 9): a greenish brown color develops.

Water Not more than 1.0 % (1.0 g, volumetric titration, direct titration).

Assay Proceed as directed in the Assay under Tetracycline Hydrochloride. Weigh accurately an amount of Tetracycline Hydrochloride Ointment, equivalent to about 30 mg (potency) according to the labeled potency, transfer to a separatory funnel, add 50 mL of ether, shake vigorously, extract 3 times with 20 mL, 10 mL, and 10 mL of 0.01 mol/L hydrochloric acid TS, combine the extracts, and add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet a suitable volume of this solution, dilute with the mobile phase to make the same concentration as the standard solution, and use this solution as the test solution.

Containers and Storage ***Containers***—Tight containers.

Tetrahydrozoline Hydrochloride



and enantiomer

$C_{13}H_{16}N_2 \cdot HCl$: 236.74

2-(1,2,3,4-Tetrahydronaphthalen-1-yl)-4,5-dihydro-1*H*-imidazolehydrochloride [*522-48-5*]

Tetrahydrozoline Hydrochloride contains not less than 98.0 % and not more than 100.5 % of tetrahydrozoline hydrochloride ($C_{13}H_{16}N_2 \cdot HCl$), calculated on the dried basis.

Description Tetrahydrozoline Hydrochloride is a white solid and is odorless.
Tetrahydrozoline Hydrochloride is freely soluble in water or in ethanol (95), very slightly soluble in chloroform and practically insoluble in ether.

Melting point—About 256 °C(with decomposition).

Identification (1) Determine the absorption spectra of solutions of Tetrahydrozoline Hydrochloride and Tetrahydrozoline Hydrochloride RS (1 in 4000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities at the same wavelengths and the respective absorbances, calculated on the dried basis, at the wavelengths of a maximum absorbance near 264 nm and near 271 nm do not differ by more than 4.0 %.

(2) Determine the infrared spectra of Tetrahydrozoline Hydrochloride and Tetrahydrozoline Hydrochloride RS, previously dried at 105 °C for 2 hours, as directed in the potassium bromide disk method under Infrared spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Tetrahydrozoline Hydrochloride (1 in 200) responds to the Qualitative Tests for chloride.

Purity (1) ***Heavy metals***—Weigh 0.40 g of Tetrahydrozoline Hydrochloride, dissolve in 23 mL of water, add 2 mL of 1 mol/L acetic acid TS and perform the test according to the Method 1. Prepare the control solution with 2.0 mL of standard lead solution (not more than 50 ppm).

(2) ***Related substances***—Weigh 0.1 g of Tetrahydrozoline Hydrochloride in 10 mL of methanol and use this solution as the test solution. Separately, weigh 10 mg of Tetrahydrozoline Hydrochloride RS, previously dried at 105 °C for 2 hours and dissolve in methanol to make exactly 10 mL. Pipet 0.1 mL, 0.5 mL, 1 mL and 2 mL of this solution, add methanol to each solution to make exactly 10 mL and use these solutions as the standard solutions (1), (2), (3) and (4), respectively. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 20 μL each of the test solution and standard solutions (1), (2), (3) and (4) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, acetic acid (100) and water (8 : 1 : 1) to a distance of about 15 cm and air-dry the plate. Spray evenly hexachloroplatinic (IV) acid-potassium iodide TS on the plate and examine under ultraviolet light (254 nm and 366 nm): intensity of the spots other than the principal spot from the test solution is not more than 2.0 % of the spot from the standard solution.

Loss on Drying Not more than 1.0 % (1 g, 105 °C, 2 hours)

Residues on Ignition Not more than 0.1 % (1 g)

Assay Weigh accurately about 0.4 g of Tetrahydrozoline Hydrochloride, transfer to a beaker and add 60 mL of acetic acid (100). Dissolve the solution by warming, if necessary. Add 5 mL of acetic anhydride and 5 mL of mercury (II) acetate and titrate

with 0.1 mol/L of perchloric acid VS (indicator: 3 drops of quinaldine red reagent). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 23.674 mg of $C_{13}H_{16}N_2 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

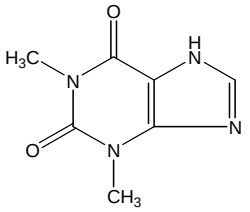
Thallium (^{201}Tl) Chloride Injection

Thallium (^{201}Tl) Chloride Injection is an aqueous solution for injection containing Thallium-201 in the form of thallous chloride.
Thallium (^{201}Tl) Chloride Injection conforms to the requirements of Thallium (^{201}Tl) Chloride Injection in the Korean Pharmaceutical Codex.
The Insoluble Particulate Matter Test for Injections is not applied to this injection.

Description Thallium (^{201}Tl) Chloride Injection is a clear, colorless liquid.

Bacterial Endotoxins Less than 175/V EU/mL of Thallium (^{201}Tl) Chloride Injection, where V is the maximum recommended dose per mL during the effective time.

Theophylline



$C_7H_8N_4O_2$: 180.16

1,3-Dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione
[*58-55-9*]

Theophylline, when dried, contains not less than 99.0 % and not more than 101.0 % of theophylline ($C_7H_8N_4O_2$).

Description Theophylline appears as white crystals or crystalline powder.
Theophylline is soluble in *N,N*-dimethylformamide, and slightly soluble in water or in ethanol (99.5).
Theophylline dissolves in 0.1 mol/L hydrochloric acid TS.

Identification (1) Determine the absorption spectra of solutions of Theophylline and Theophylline RS in 0.1moL/L hydrochloric acid TS (1 in 200000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Theophylline and Theophylline RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 271 ~ 275 °C.

Purity (1) ***Acid***—To 0.5 g of Theophylline, add 75 mL of water, 2.0 mL of 0.01 mol/L sodium hydroxide VS and 1 drop of methyl red TS: a yellow color is observed.

(2) ***Heavy metals***—Proceed with 1.0 g of Theophylline according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Theophylline according to Method 3 and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 0.10 g of Theophylline in 3 mL of *N,N*-dimethylformamide, add 10 mL of methanol and use this solution as the test solution. Pipet 1.0 mL of the test solution, add methanol to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography, Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone, chloroform, methanol, 1-butanol and ammonia solution (28) (3 : 3 : 2 : 2 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.25 g of Theophylline, previously dried, dissolve in 100 mL of water, add exactly 20 mL of 0.1 mol/L silver nitrate VS, shake the mixture and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry), Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 18.016 mg of $C_7H_8N_4O_2$

Containers and Storage ***Containers***—Well-closed containers.

Theophylline Tablets

Theophyllin Tablets contains not less than 94.0 % and not more than 106.0 % of the labeled amount of theophyllin ($C_7H_8N_4O_2$: 180.16).

Method of Preparation Prepare as directed under Tablets, with Theophylline.

Identification (1) Weigh a quantity of powdered Tablets, equivalent to about 0.5 g of theophylline, triturate with 10 mL and 5 mL volumes of hexane and discard the hexane. Triturate the residue with two 10 mL volumes of a mixture of ammonium hydroxide TS and water (1 : 1) and filter each time. Evaporate the combined filtrates to about 15 mL, neutralize, if necessary, with 6 mol/L acetic acid TS, using litmus and then cool to about 15 °C, with stirring. Collect the precipitate on a filter, wash it with cold water and dry at 105 °C for 2 hours: the residue so obtained melts between 270 °C and 274 °C.

(2) Determine the infrared spectra of the residue obtained in Identification (1) and Theophylline RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) The retention time of the main peak obtained as directed in the Assay from the test solution corresponds to that exhibited by the standard solution.

Dissolution Test Perform the test with 1 tablet of Theophylline Tablets at 50 revolutions per minute according to Method 2 under the dissolution test, using 900 mL of water as the dissolution solution. Take a portion of dissolved solution 45 minutes after start of the test, filter, dilute with a portion of test solution, if necessary and use this solution as the test solution. Separately, weigh accurately a portion of Theophylline RS, previously dried at 105 °C for 4 hours, prepare the solution according to the same method as the test solution and use this solution as the standard solution. Determine the absorbances of the test solution and the standard solution at 272 nm as directed under Ultraviolet-visible Spectrophotometry, using the dissolution solution as blank.

The dissolution rate of Theophylline Tablets in 45 minutes is not less than 80 %.

Uniformity of Dosage Units It meets the requirement.

Assay To 10 tablets of Theophylline Tablets, add 50 mL of water and 50 mL of ammonium hydroxide TS, disintegrated the tablets completely by shaking well, add water to make exactly 500 mL and filter through a dry filter. Discard the first 20 mL of the filtrate, pipet a portion of the subsequent filtrate (V mL), equivalent to about 10 mg of theophylline ($C_7H_8N_4O_2$) according to the labeled amount, add 20 mL of internal standard solution, dilute with the mobile phase to make exactly 100 mL and use this solution as the test solution. Separately, weigh accurately a portion of theophylline RS, previously dried at 105 °C for 4 hours and dissolve in mobile phase so that each mL contains 1 mg of theophylline. Pipet 10 mL of this solution, add 20.0 mL of the internal standard solution, dilute with the mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the ratios of peak area, Q_T and Q_S of theophylline for each solution to that of the internal standard.

$$\text{Amount (mg) of theophyllin } (C_7H_8N_4O_2) = 5000 \times \frac{C}{V} \times \frac{Q_T}{Q_S}$$

C: Concentration (mg/mL) of theophylline in the standard solution,

Internal standard solution—Weigh accurately about 50 mg of theobromine, dissolve in 10 mL of ammonium hydroxide TS and add mobile phase to make exactly 100 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography.

Mobile phase: 70 mL of acetonitrile in buffer to make 1000 mL.

Flow rate: 1 mL/minute.

System suitablilty

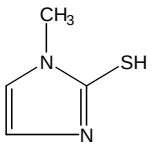
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the resolution between internal standard and theophylline is not less than 1.5.

System repeatability: When the test repeated 6 times with 20 μL each of standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of theophylline to that of the internal standard is not more than 1.5 %.

Buffer solution—Dissolve 2.72 g of sodium acetate trihydrate in 200 mL of water, add 20 mL of acetic acid (100) and add water to make 2 L.

Containers and Storage ***Containers***—Well-closed containers.

Thiamazole



$C_4H_6N_2S$: 114.17

3-Methyl-1H-imidazole-2-thione [*60-56-0*]

Thiamazole, when dried, contains not less than 98.0 % and not more than 101.0 % of thiamazole ($C_4H_6N_2S$).

Description Thiamazole appears as white to pale yellowish white crystals or crystalline powder, has a faint, characteristic odor and has a bitter taste.
Thiamazole is freely soluble in water or in ethanol (95) and slightly soluble in ether.

pH—Dissolve 1.0 g of Thiamazole in 50 mL of water: the pH of this solution is between 5.0 and 7.0.

Identification (1) Dissolve 5 mg of Thiamazole in 1 mL of water, shake with 1 mL of sodium hydroxide TS and add 3 drops of sodium pentacyanonitrosylferrate (III) TS: a yellow color is observed and it gradually changes to yellow-green to green. To this solution, add 1 mL of acetic acid (31): it changes to blue.

(2) To 2 mL of a solution of Thiamazole (1 in 200), add 1 mL of sodium carbonate TS and 1 mL of diluted Folin's TS (1 in 5): a deep blue color is observed.

Melting Point 144 ~ 147 °C.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Thiamazole according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Selenium***—Proceed with 0.10 g of Thiamazole as directed under the Oxygen Flask Combustion Method, using 25 mL of diluted nitric acid (1 in 30) as the absorbing liquid and prepare the test solution. Apply a small volume of water to the upper part of apparatus A, pull out C carefully and transfer the test solution to a beaker. Wash C, B and the inner side of A with 25 mL of water and combine the washings with the test solution. Boil gently for 10 minutes, cool to room temperature, add water to make exactly 50 mL and use this solution as the test solution. Separately, weigh exactly 40 mg of selenium, dissolve in 100 mL of diluted nitric acid (1 in 2), heat to dissolve on a water-bath, if necessary and add water to make exactly 1000 mL. Pipet 1 mL of this solution and add water to make exactly 200 mL. To 2 mL of this solution, exactly measured, add diluted nitric acid (1 in 60) to make exactly 50 mL and use this solution as the standard solution. Pipet 40 mL each of the test solution and the standard solution into separate beakers and adjust each solution with ammonia solution (28) to a pH of 1.8 to 2.2. To each solution, add 0.2 g of hydroxylamine hydrochloride, shake gently to dissolve, then add 5 mL of 2,3-diaminonaphthalene TS, shake and allow to stand for 100 minutes. Transfer these solutions to corresponding separatory funnels, rinse the beakers with 10 mL of water, combine the rinsings in the respective separatory funnels, shake well with 5.0 mL of cyclohexane for 2 minutes and extract. Centrifuge the cyclohexane extracts to remove any water remaining in these solutions. Perform the test with the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry, using a solution prepared with 40 mL of diluted nitric acid (1 in 60) in the same manner as the blank. The absorbance of the test solution at the wavelength of maximum absorbance near 378 nm does not exceed the absorbance of the standard solution.

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Thiamazole according to Method 1 and perform the test (not more than 2 ppm).

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.25 g of Thiamazole, previously dried, dissolve in 75 mL of water, add 15.0 mL of 0.1 mol/L sodium hydroxide VS from a burette and add 30 mL of 0.1 mol/L silver nitrate VS with stirring. Add 1 mL of bromothymol blue TS and titrate with 0.1 mol/L sodium hydroxide VS, until a persistent blue-green color is observed. Determine the total volume of 0.1 mol/L sodium hydroxide VS consumed.

Each mL of 0.1 mol/L sodium hydroxide VS
= 11.417 mg of $C_4H_6N_2S$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Thiamazole Tablets

Thiamazole Tablets contain not less than 94.0 % and not more than 106.0 % of the labeled amount of thiamazole ($C_4H_6N_2S$: 114.17).

Method of Preparation Prepare as directed under Tablets, with Thiamazole.

Identification (1) To a quantity of powdered Thiamazole Tablets, equivalent to 50 mg of thiamazole according to the labeled amount, add 20 mL of hot ethanol, shake for 15 minutes, filter and evaporate the filtrate on a water-bath to dryness. Dissolve the residue in 10 mL of water, filter, if necessary and use this solution as the test solution. To 1 mL of the test solution, add 1 mL of sodium hydroxide TS, shake and add 3 drops of sodium nitroprusside TS: a yellow color de-

velops and it gradually changes to yellow-green to green. To this solution, add 1 mL of acetic acid (31): it changes to blue.

(2) With 2 mL of the test solution obtained in (1), proceed as directed in the Identification (2) under Thiamazole.

Dissolution Test Perform the test with 1 tablet of Thiamazole Tablets at 100 revolutions per minute according to Method 1, using 500 mL of water as the dissolution solution. Take the dissolved solution 30 minutes after the start of the test, and filter. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution solution to make exactly V' mL, and use this solution as the test solution. Separately, weigh accurately a suitable amount of Thiamazole RS, dissolve in the dissolution solution to make the same concentration as the test solution, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 252 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry. The dissolution rate of Thiamazole Tablets in 30 minutes is not less than 80 % (Q).

Dissolution rate (%) with respect to the labeled amount of thiamazole ($C_4H_6N_2S$)

$$= C_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 50000$$

C_S: Concentration (mg/mL) of the standard solution
C: Labeled amount (mg) of thiamazole ($C_4H_6N_2S$) in 1 tablet

Uniformity of Dosage Units It meets the requirement.

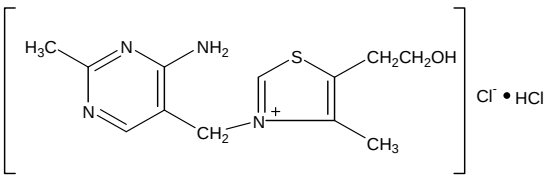
Assay Weigh accurately and powder not less than 20 Thiamazole Tablets. Weigh accurately a quantity of the powder, equivalent to about 0.15 g of thiamazole ($C_4H_6N_2S$), add 80 mL of water, shake for 15 minutes, add water to make exactly 100 mL and centrifuge. Filter, discard the first 20 mL of the filtrate, take exactly 50 mL of the subsequent filtrate, add 1 mL of bromothymol blue TS and if a blue color develops, neutralize with 0.1 mol/L hydrochloric acid VS until the color of the solution changes to green. To this solution, add 4.5 mL of 0.1 mol/L sodium hydroxide VS from a burette, add 15 mL of 0.1 mol/L silver nitrate VS while stirring and titrate with 0.1 mol/L sodium hydroxide VS. Continue the titration until a persistent blue-green color is produced and determine the total volume of 0.1 mol/L sodium hydroxide VS consumed.

Each mL of 0.1 mol/L sodium hydroxide VS
= 11.417 mg of $C_4H_6N_2S$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Thiamine Hydrochloride



Vitamin B_1 Hydrochloride

$C_{12}H_{17}ClN_4OS \cdot HCl$: 337.27

3-[(4-Amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methyl-1,3-thiazol-3-ium chloride hydrochloride [*67-03-8*]

Thiamine Hydrochloride contains not less than 98.5 % and not more than 101.0 % of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$), calculated on the anhydrous basis.

Description Thiamine Hyrochloride appears as white crystals or crystalline powder and is odorless or has a slight characteristic odor.
Thiamine Hydrochloride is freely soluble in water, sparingly soluble in methanol, slightly soluble in ethanol (95) and practically insoluble in ether.

Melting point—About 245 °C (with decomposition).

Identification (1) To 5 mL of a solution of Thiamine Hydrochloride (1 in 500), add 2.5 mL of sodium hydroxide TS and 0.5 mL of potassium hexacyanoferrate (III) TS. Then add 5 mL of 2-methyl-1-propanol, shake the mixture vigorously for 2 minutes, allow to stand and examine under ultraviolet light (main wavelength: 365 nm): the 2-methyl-1-propanol layer shows a blue-purple fluorescence. This fluorescence disappears when the mixture is acidified, but reappears when made alkaline again.

(2) Determine the absorption spectra of Thiamine Hydrochloride and Thiamine Hydrochloride RS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar absorption at same wavelengths.

(3) Determine the infrared spectra of Thiamine Hydrochloride and Thiamine Hydrochloride RS, previously dried at 105 °C for 2 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any difference appears between the spectra, dissolve each in water, evaporate water to dryness, dry at 105 °C for 2 hours and repeat the test on the residues.

(4) A solution of Thiamine Hydrochloride (1 in 500) responds to the Qualitative Tests for chloride.

pH Dissolve 1.0 g of Thiamine Hydrochloride in 100

mL of water: the pH of this solution is between 2.7 and 3.4.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Thiamine Hydrochloride in 10 mL of water: the solution is clear and has no more color than the following control solution.

Control solution—To 1.5 mL of 1/60 mol/L potassium bichromate VS, add water to make 1000 mL.

(2) ***Sulfate***—Weigh 1.5 g of Thiamine Hydrochloride and perform the test. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.011 %).

(3) ***Nitrate***—Dissolve 0.5 g of Thiamine Hydrochloride in 25 mL of water. Add 2 mL of sulfuric acid to 2 mL of this solution, shake, cool and superimpose iron (II) sulfate TS: no dark brown ring is observed at the junction of the two layers.

(4) ***Heavy metals***—Proceed with 1.0 g of Thiamine Hydrochloride according to Method 1 and perform the test. Prepare the control solution: with 2.0 mL of standard lead solution (not more than 20 ppm).

(5) ***Related substances***—Dissolve 0.10 g of Thiamine Hydrochloride in 100 mL of the mobile phase and use this solution as the test solution. Pipet 1 mL of the test solution, add the mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and determine the area of each peak from these solutions by the automatic integration method: total area of the peaks other than the principal peak is not larger than the peak area of the principal peak from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, flow rate and selection of column: Proceed as directed in the operating conditions in the Assay.

System suitability

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: To exactly 5 mL of the standard solution add water to make exactly 50 mL. Confirm that the peak area of thiamine obtained from l0 μL of this solution is equivalent to 7 to 13 % of that of thiamine obtained from l0 μL of the standard solution.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of thiamine is not more than 1.0 %.

Time span of measurement: About 3 times of the retention time of thiamine.

Water Not more than 5.0 % (30 mg, coulometric titration).

Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 0.1 g each of Thiamine Hydrochloride and Thiamine Hydrochloride RS (water content is determined previously) and dissolve the mobile phase to make exactly 50 mL. Pipet 10 mL each of the solutions, add exactly 5 mL of the internal standard solution, then add the mobile phase to make exactly 50 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and calculate the ratios, Q_T and Q_S, of the peak area of thiamine to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of thiamine hydrochloride } (C_{12}H_{17}ClN_4OS\cdot HCl) = \text{Amount (mg) of Thiamine Hydrochloride RS, calculated on the anhydrous basis} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of methyl benzoate in methanol (1 in 50).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and 15 cm to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5μm to 10 μm in particle diameter).

Column temperature: A constant temperature of about 25 ° C.

Mobile phase: Dissolve 1.1 g of sodium 1-octanesulfonate in 1000 mL of diluted acetic acid (100) (1 in 100). To 600 mL of this solution, add 400 mL of a mixture of methanol and acetonitrile (3 : 2).

Flow rate: Adjust the flow rate so that the retention time of thiamine is about 12 minutes.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution according to the above operating conditions, thiamine and the internal standard are eluted in this order with the resolution between their peaks being not less than 6.0.

System repeatability: When the test repeated 6 times with 10 μL each of standard solution under the above operating conditions, the relative standard deviation of the ratio of peak area of thiamine to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Thiamine Hydrochloride Injection

Vitamin B_1 Hydrochloride Injection

Thiamine Hydrochloride Injection is an aqueous solution for injection. Thiamine Hydrochloride Injection contains not less than 95.0 % and not more than 115.0 % of the labeled amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$: 337.27).

Method of Preparation Prepare as directed under Injections, with Thiamine Hydrochloride.

Description Thiamine Hydrochloride Injection is a clear, colorless liquid.

pH—2.5 ~ 4.5.

Identification To a volume of Thiamine Hydrochloride Injection, equivalent to 50 mg of thiamine hydrochloride according to the labeled amount, add water to make 25 mL. Proceed with 5 mL of this solution as directed in the Identification (1) under Thiamine Hydrochloride.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 6.0 EU/mg of thiamine hydrochloride.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay Dilute with 0.001 mol/L hydrochloric acid TS, if necessary, then measure exactly a volume of Thiamine Hydrochloride Injection, equivalent to about 20 mg of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) and add 20 mL of methanol and 0.001 mol/L hydrochloric acid TS to make 100 mL. To exactly 25 mL of this solution, add exactly 5 mL of the internal standard solution and 0.001 mol/L hydrochloric acid TS to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately about 0.1 g of Thiamine Hydrochloride RS (water content is determined previously) and dissolve in 0.001 mol/L hydrochloric acid TS to make exactly 50 mL. To exactly 10 mL of this solution, add 20 mL of methanol and 0.001 mol/L hydrochloric acid TS to make exactly 100 mL. To exactly 25 mL of this solution, add exactly 5 mL of the internal standard solution and 0.001 mol/L hydrochloric acid TS to make exactly 50 mL and use this solution as the standard solution. Proceed as directed in the Assay under Thiamine Hydrochloride.

$$\text{Amount (mg) of } C_{12}H_{17}ClN_4OS \cdot HCl = \text{Amount (mg) of Thiamine Hydrochloride RS, calculated on the anhydrous basis} \times \frac{Q_T}{Q_S} \times \frac{1}{5}$$

Internal standard solution—A solution of methyl benzoate in methanol (1 in 200).

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Thiamine Hydrochloride Tablets

Thiamine Hydrochloride Tablets contain not less than 90.0 % and not more than 110.0 % of the labeled amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$: 337.27).

Method of Preparation Prepare as directed under the Tablets, with Thiamine Hydrochloride.

Identification (1) To a quantity of powdered Thiamine Hydrochloride Tablets, equivalent to 10 mg of thiamine hydrochloride according to the labeled amount, add 10 mL of 0.5 mol/L of sodium hydroxide TS, shake well and filter. Perform the test with 5 mL of the filtrate according to the Identification (2) of Thiamine Hydrochloride.

(2) To a quantity of powdered Thiamine Hydrochloride Tablets, equivalent to 10 mg of thiamine hydrochloride according to the labeled amount, add 10 mL of water and filter. To 2 mL of the filtrate, add each of iodine TS and mercuric chloride TS: a red-brown and white precipitate is produced respectively.

(3) To the filtrate of (2), add 1 mL of lead acetate TS and 1 mL of 2.5 mol/L sodium hydroxide TS: a yellow color appears. Heat on the steam-bath for a few minutes: a color is changed to brown and the lead sulfate precipitate is separated.

(4) The filtrate of (2) responds to the Qualitative Tests for chloride.

Dissolution Test Perform the test with 1 tablet of Thiamine Hydrochloride Tablets at 50 revolution per minute according to Method 2 under the Dissolution Test, using 900 mL of water as the dissolution solution. Take a portion of dissolved solution 45 minutes after start of the test and use this solution as the test solution. Perform the test, as directed in the Assay. If necessary, perform the test using the standard solution, prepared from a certain amount, weighed accurately, by the same manner with the test solution.

The dissolution rate of Thiamine Hydrochloride Tablets in 45 minutes is not less than 75.0 %.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately and powder not less than 20 Thiamine Hydrochloride Tablets. Weigh accurately a portion of the powder, equivalent to about 20 mg of thiamine hydrochloride, add 60 mL of 0.01 mol/L hydrochloric acid TS, heat in a water bath for 30 minutes, shake vigorously for 10 minutes, cool, add methanol to make exactly 100 mL, and centrifuge. Pipet 25 mL of the clear supernatant liquid, add exactly 5 mL of the internal standard solution, add water to make 50 mL, and use this solution as the test solution. Separately, weigh accurately about 0.1 g of Thiamine Hydrochloride RS (separately determine the water in the same manner as Thiamine Hydrochloride), and dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 10 mL of this solution, add 50 mL of 0.01 mol/L hydrochloric acid TS, and add methanol to make exactly 100 mL. Pipet 25 mL of this solution, add exactly 5 mL of the internal standard solution and water to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Thiamine Hydrochloride.

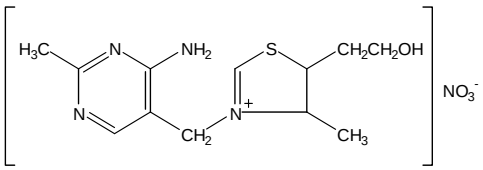
Amount (mg) of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$)
= Amount (mg) of Thiamine Hydrochloride RS,
calculated on the anhydrous basis $\times \frac{Q_T}{Q_S} \times \frac{1}{5}$

Internal standard solution—A solution of methyl benzoate in methanol (1 in 200)

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Thiamine Nitrate



Vitamin B_1 Nitrate $C_{12}H_{17}N_5O_4S$: 327.36

3-[(4-Amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methyl-1,3-thiazol-3-ium chloride nitrate [*532-43-4*]

Thiamine Nitrate, when dried, contains not less than 98.0 % and not more than 102.0 % of thiamine nitrate ($C_{12}H_{17}N_5O_4S$).

Description Thiamine Nitrate appears as white crystals or crystalline powder and is odorless or has a slight, characteristic odor.

Thiamine Nitrate is sparingly soluble in water and very slightly soluble in ethanol (95) and practically insoluble in ether.

Melting point—About 193 °C (with decomposition).

Identification (1) Take 2 mL of a solution of Thiamine Nitrate (1 in 500) and add 2 to 3 drops of iodine TS: a red-brown precipitate or turbidity is produced. Upon further addition of 1 mL of 2,4,6-trinitrophenol TS; a yellow precipitate or turbidity is produced.

(2) To 1 mL of a solution of Thiamine Nitrate (1 in 500), add 1 mL of lead acetate TS and 1 mL of a solution of sodium hydroxide (1 in 10) and warm: the color of the solution changes through yellow to brown and on standing, a black-brown precipitate is produced.

(3) To 5 mL of a solution of Thiamine Nitrate (1 in 500), add 2.5 mL of sodium hydroxide TS and 0.5 mL of potassium hexacyanoferrate (III) TS. Then add 5 mL of 2-methyl-1-propanol, shake the mixture vigorously for 2 minutes, allow to stand and examine under ultraviolet light (main wavelength: 365 nm): the 2-methyl-1-propanol layer shows a blue-purple fluorescence. This fluorescence disappears when the mixture is acidified, but reappears when it is made alkaline again.

(4) A solution of Thiamine Nitrate (1 in 50) responds to the Qualitative Tests (1) and (2) for nitrate.

pH Dissolve 1.0 g of Thiamine Nitrate in 100 mL of water: the pH of this solution is between 6.5 and 8.0.

Purity (1) ***Chloride***—Perform the test with 0.20 g of Thiamine Nitrate. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.053 %).

(2) ***Sulfate***—Dissolve 1.5 g of Thiamine Nitrate in 30 mL of water and 2 mL of dilute hydrochloric acid and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS and 2 mL of dilute hydrochloric acid and add water to make 50 mL (not more than 0.011 %).

(3) ***Heavy metals***—Dissolve 1.0 g of Thiamine Nitrate in 30 mL of water by warming, cool and add 12 mL of 6 mol/L acetic acid TS and water to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Related substances***—Weigh accurately 0.1 g of Thiamine Nitrate, dissolve in the mobile phase to make 100 mL, and use this solution as the test solution. Perform the test with 10 μL of the test solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas of this solution by the automatic integration method: the total area of the peaks other than the principal peak from the test solution is not more than 1.0 % of the total area of all peaks from the test solution.

Operating conditions

Detector, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay.

Column: A stainless steel column about 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Flow rate: 0.75 mL/minute

Time span of measurement: About 3 times as long as the retention time of thiamine.

Loss on Drying Not more than 1.0 % (0.5 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 0.1 g each of Thiamine Nitrate, previously dried, and Thiamine Hydrochloride RS (water content is determined previously) and dissolve each of them in the mobile phase to make exactly 50 mL. Pipet 10 mL each of the solutions, add exactly 5 mL each of the internal standard solution, add the mobile phase to make exactly 50 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of thiamine to that of the internal standard.

Amount (mg) of thiamine nitrate ($C_{12}H_{17}N_5O_4S$)
= Amount (mg) of Thiamine Hydrochloride RS,
calculated on the anhydrous basis $\times \frac{Q_T}{Q_S} \times 0.9706$

Internal standard solution—A solution of methyl benzoate in methanol (1 in 50).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter)

Column temperature: A constant temperature of about 30 °C.

Mobile phase: Dissolve 1.1 g of sodium 1-octanesulfonate in 1000 mL of diluted acetic acid (100) (1 in 100). To 600 mL of this solution add 400 mL of a mixture of methanol and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of thiamine is about 12 minutes.

System suitability

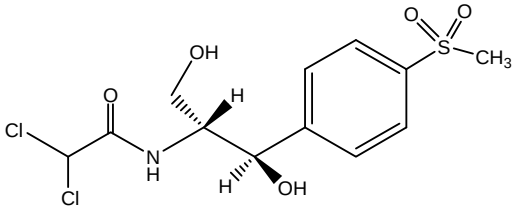
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, thiamine and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of thiamine to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Thiamphenicol



$C_{12}H_{15}Cl_2NO_5S$: 356.22

2,2-Dichloro-*N*-[(1*R*,2*R*)-1,3-dihydroxy-1-(4-methylsulfonylphenyl)propan-2-yl]acetamide
[*15318-45-3*]

Thiamphenicol contains not less than 980 μg (potency) per mg of thiamphenicol ($C_{12}H_{15}Cl_2NO_5S$: 356.22), calculated on the anhydrous basis.

Description Thiamphenicol appears as white to yellowish white crystals or crystalline powder.
Thiamphenicol is very soluble in dimethylacetamide, freely soluble in *N,N*-dimethylformamide or in acetonitrile, sparingly soluble in ethanol (95) or in acetone, and slightly soluble in water, in ether, or in ethyl acetate.

Identification (1) Determine the infrared spectra of Thiamphenicol and Thiamphenicol RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Preare a 1 % (potency) solution of Thiamphenicol in methanol, and use this solution as the test solution. Separately, prepare a 1 % (potency) solution of Thiamphenicol RS in methanol, and use this solution as the standard solution. Perform the test with the test solution and standard solution as directed under Thin-layer Chromatography. Spot the test solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and methanol (97 : 3), and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots obtained from the test solution and standard solution have the same R_f value.

(3) To 5 mL of a 0.1 % (potency) solution of Thiamphenicol add 0.2 mL of silver nitrate TS: no precipitate is produced. To 50 mg of Thiamphenicol add 2 mL of potassium hydroxide-ethanol TS, warm in a water bath for 15 minutes, add 15 mg of activated carbon to decolorize, shake, and filter. The filtrate responds to the Qualitative Tests for chloride.

Specific Optical Rotation $[\alpha]_D^{20}$: -21 ~ -24° (1.25 g, dimethylformimide, 25 mL, 100 mm).

Melting Point 163 ~ 167 °C.

Absorbance Weigh accurately about 20 mg (potency) of Thiamphenicol, add 60 mL of water, dissolve by warming at 40 °C, add water to make exactly 100 mL, and use this solution as the test solution. Determine the absorption spectrum of the test solution as directed under Ultraviolet-visible Spectrophotometry: $E_{1cm}^{1\%}$ at about 266 nm and 273 nm are 25 to 28 and 21.5 to 23.5, respectively. Determine the absorption spectrum of a solution of the test solution (1 in 20) as directed under Ultraviolet-visible Spectrophotometry: $E_{1cm}^{1\%}$ at about 224 nm is 370 to 400.

Purity (1) ***Acid or alkali***—To 0.1 g (potency) of Thiamphenicol add 20 mL of freshly boiled and cooled water, shake, add 0.1 mL of bromothymol blue TS, and neutralize with 0.02 mol/L hydrochloric acid solution or 0.02 mol/L sodium hydroxide solution until the color of the solution disappears: not more than 0.1 mL is consumed.

(2) ***Chloride***—Weigh accurately about 0.5 g (potency) of Thiamphenicol, add 30 mL of water, shake for 5 minutes, filter, and perform the test with 15 mL of the filtrate. Prepare the control solution with 0.14 mL of 0.01 mol/L hydrochloric acid TS (not more than 0.20 %).

(3) ***Heavy metals***—Proceed with 1.0 g of Thiamphenicol according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

Loss on Drying Not more than 1.0 % (1.0 g, 60 °C, 3 hours).

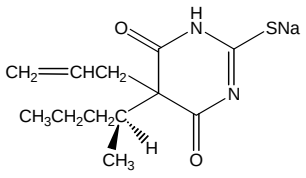
Residue on Ignition Not more than 0.1 % (2 g).

Assay Weigh accurately about 50 mg (potency) each of Thiamphenicol and Thiamphenicol RS, dissolve in ethanol (95) to make exactly 100 mL, and use these solutions as the test solution and standard solution, respectively. Pipet 2.0 mL each of the test solution and standard solution, transfer to 100 mL volumetric flasks, add ethanol (95) to make exactly 100 mL, and determine the absorbances, A_T and A_S, at 225 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry, using ethanol (95) as the blank.

$$\text{Amount [μg (potency)] of thiamphenicol} = \text{Amount [μg (potency)] of Thiamphenicol RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Tight containers.

Thiamylal Sodium



and enantiomer

$C_{12}H_{17}N_2NaO_2S$: 276.33

Sodium 5-allyl-6-oxo-5-(2-pentanyl)-2-thioxo-1,2,5,6-tetrahydro-4-pyrimidinolate [*337-47-3*]

Thiamylal Sodium contains not less than 97.5 % and not more than 101.0 % of thiamylal sodium ($C_{12}H_{17}N_2NaO_2S$), calculated on the dried basis.

Description Thiamylal Sodium appears as pale yellow crystals or powder.
Thiamylal Sodium is very soluble in water, and freely soluble in ethanol (95).

pH—Dissolve 1.0 g of Thiamylal Sodium in 10 mL of water: the pH of this solution is between 10.0 and 11.0.
Thiamylal Sodium is hygroscopic.
Thiamylal Sodium is gradually decomposed by light.
A solution of Thiamylal Sodium in ethanol (95) (1 in 10) shows no optical rotation.

Identification (1) Determine the absorption spectra of solutions of Thiamylal Sodium and Thiamylal Sodium RS in ethanol (7 in 1000000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Thiamylal Sodium and Thiamylal Sodium RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotomctry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Thiamylal Sodium (1 in 10) responds to the Qualitative Tests (1) and (2) for sodium salt.

Purity (1) ***Clarity and color of solution***—To 1.0 g of Thiamylal Sodium in a 11 to 13 mL glass-stoppered test tube, add 10 mL of freshly boiled and cooled water, stopper tightly, allow to stand and dissolve by occasional gentle shaking: the solution is clear and pale

yellow.

(2) ***Heavy metals***—Proceed with 1.0 g of Thiamylal Sodium according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Dissolve about 0.10 g of Thiamylal Sodium in 10 mL of ethanol (95), and use this solution as the test solution. Pipet 1 mL and 3 mL. of the test solution, add ethanol (95) to make exactly 200 mL, and use these solutions as the standard solution (1) and the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography, develop with a mixture of toluene, methanol and ethyl acetate (40 : 7 : 3) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand in iodine vapor overnight: the spot appeared with R_f of about 0.1 obtained with the test solution is not more intense than the spot with the standard solution (2), and the spot other than the principal spot, the spot at origin and the spot mentioned above obtained with the test solution is not more intense than the spot with the standard solution (1).

Loss on Drying Not more than 2.0 % (1 g, 105 °C, 1 hour).

Assay Weigh accurately about 0.25 g of Thiamylal Sodium, dissolve in 50 mL of methanol and 5 mL of dilute hydrochloric acid, and add methanol to make exactly 100 mL. Pipet 10 mL of this solution, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and the mobile phase to make exactly 200 mL, and use this solution as the test solution. Separately, weigh accurately about 23 mg of Thiamylal RS, previously dried at 105 °C for 1 hour, dissolve in 50 mL of methanol and 0.5 mL of dilute hydrochloric acid, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and the mobile phase to make exactly 200 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of thiamylal to that of the internal standard.

$$\text{Amount (mg) of thiamylal sodium } (C_{12}H_{17}N_2NaO_2S) = \text{Amount (mg) of Thiamylal RS} \times \frac{Q_T}{Q_S} \times 10 \times 1.0864$$

Internal standard solution—A solution of phenyl benzoate in methanol (3 in 500).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 289 nm).

Column: A stainless steel column about 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanised silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: A mixture of methanol and 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.6 (13:7).

Flow rate: Adjust the flow rate so that the retention time of thiamylal is about 6 minutes.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, thiamylal and the internal standard are eluted in this order with the resolution between these peaks being not less than 12.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of thiamylal to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Thiamylal Sodium for Injection

Thiamylal Sodium for Injection is a preparation for injection which is reconstituted before use. Thyamylal Sodium for Injection contains not less than 93.0 % and not more than 107.0 % of the labeled amount of thiamylal sodium ($C_{12}H_{17}N_2NaO_2S$).

Method of Preparation Prepare as directed under Injections, with 100 parts of Thiamylal Sodium and 7 parts by mass of Dried Sodium Carbonate.

Description Thiamylal Sodium for Injection appears as pale yellow crystals, powder or mass.
Thiamylal Sodium for Injection is hygroscopic.
Thiamylal Sodium for Injection is gradually decomposed by light.

Identification (1) To 1.0 g of Thiamylal Sodium for Injection add 20 mL of ethanol (95), shake vigorously, and filter. Dissolve the residue in 1 mL of water, and add 1 mL of barium chloride TS: a white precipitate is produced. Centrifuge this solution, carefully remove the clear supernatant liquid, and to the precipitate add dilute hydrochloric acid dropwise: the precipitate dissolves with effervescence.

(2) To 50 mg of Thiamylal Sodium for Injection add 100 mL of ethanol (95), shake vigorously, and filter. To 3 mL of the filtrate add ethanol (95) to make 200 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits absorption maxima between 236 nm and 240 nm, and between 287 nm and 291 nm.

pH Dissolve 1.0 g of Thiamylal Sodium for Injection in 40 mL of water: the pH of this solution is between 10.5 and 11.5.

Purity ***Related substances***—To 0.10 g of Thiamylal Sodium for Injection add 10 mL of ethanol (95), shake vigorously, filter, and use the filtrate as the test solution. Proceed as directed in the Purity (3) under Thiamylal Sodium

Sterility Test It meets the requirement.

Bacterial Endotoxin Less than 1.0 EU/mg of Thiamylal Sodium for Injection.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay Open carefully 10 containers of Thiamylal Sodium for Injection, dissolve the contents with water, wash out the inside of each container with water, combine them, and add water to make exactly *V* mL so that each mL contains about 5 mg of thiamylal sodium ($C_{12}H_{17}N_2NaO_2S$). Pipet 5 mL of this solution, and add 0.5 mL of dilute hydrochloric acid and methanol to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and the mobile phase to make exactly 200 mL, and use this solution as the test solution. Proceed the test with the test solution as directed in the Assay under Thiamylal Sodium.

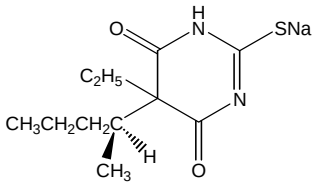
$$\text{Amount (mg) of thiamylal sodium } (C_{12}H_{17}N_2NaO_2S) = \text{Amount (mg) of Thiamylal RS} \times \frac{Q_T}{Q_S} \times \frac{V}{50} \times 1.0864$$

Internal standard solution—A solution of phenyl benzoate in methanol (3 in 500).

Containers and Storage ***Containers***—Hermetic containers.

Storage—Light-resistant.

Thiopental Sodium



$C_{11}H_{17}N_2NaO_2S$: 264.32

Sodium 5-ethyl-4,6-dioxo-5-pentan-2-yl-1*H*-pyrimidine-2-thiolate [*71-73-8*]

Thiopental Sodium, when dried, contains not less than 97.0 % and not more than 101.0 % of thiopental sodium ($C_{11}H_{17}N_2NaO_2S$).

Description Thiopental Sodium is a pale yellow powder and has a faint, characteristic odor.
Thiopental Sodium is very soluble in water, freely soluble in ethanol (95) and practically insoluble in ether.
A solution of Thiopental Sodium (1 in 10) is alkaline.
Thiopental Sodium is hygroscopic.
A solution of Thiopental Sodium gradually decomposes on standing.

Identification (1) Dissolve 0.2 g of Thiopental Sodium in 5 mL of sodium hydroxide TS and add 2 mL of lead acetate TS: a white precipitate, which dissolves upon heating, is produced. Boil the solution thus obtained: a black precipitate forms gradually and the precipitate responds to the Qualitative Tests for sulfide.

(2) Dissolve 0.5 g of Thiopental Sodium in 15 mL of water, add 10 mL of dilute hydrochloric acid to produce white precipitate and extract with four 25 mL volumes of chloroform. Combine the chloroform extracts, evaporate on a water-bath and dry at 105 °C for 2 hours: the residue melts between 157 °C and 162 °C.

(3) A solution of Thiopental Sodium (1 in 10) responds to the Qualitative Tests (1) and (2) for sodium salt.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Thiopental Sodium in 10 mL of freshly boiled and cooled water: the solution is clear and pale yellow.

(2) ***Heavy metals***—Dissolve 2.0 g of Thipental Sodium in 76 mL of water, add 4 mL of dilute hydrochloric acid, shake and filter through a glass filter (G4). To 40 mL of the filtrate, add 2 mL of ammonium acetate TS, dilute with water to make 50 mL and perform the test using this solution as the test solution. Prepare a control solution as follows: to 2.0 mL of standard lead solution, add 2 mL of dilute acetic acid, 2 mL of ammonium acetate TS and water to make 50 mL (not more than 20 ppm).

(3) ***Neutral and basic substances***—Weigh accurately about 1.0 g of Thiopental Sodium, dissolve in 10 mL of water and 5 mL of sodium hydroxide TS and shake vigorously with 40 mL of chloroform. Separate the chloroform layer, wash with two 5 mL volumes of water, filter and evaporate the filtrate on a water-bath to dryness. Dry the residue at 105 °C for 1 hour: the amount of the residue is not more than 0.50 %.

(4) ***Related substances***—Dissolve 50 mg of Thiopental Sodium in 50 mL of the mobile phase and use this solution as the test solution. Pipet 1 mL of the test solution, add the mobile phase to make exactly 200 mL and use this solution as the standard solution. Perform

the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Measure each peak area of each solution by the automatic integration method: the total area of peaks other than those of thiopental in the test solution is not larger than the peak area of thiopental in the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and 15 cm to 25 cm in length, packed with octadecylsilanized silica gel (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: Dissolve 1 g of potassium dihydrogen phosphate in 1000 mL of water and adjust with phosphoric acid to a pH of 3.0. To 700 mL of this solution, add 300 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of thiopental is about 15 minutes.

System suitablilty

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of thiopental obtained from 20 μL of this solution is equivalent to 15 to 25 % of that of thiopental obtained from 20 μL of the standard solution.

System performance: Dissolve 5 mg each of isopropyl parahydroxybenzoate and propyl parahydroxybenzoate in 50 mL of acetonitrile and add water to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, isopropyl parahydroxybenzoate and propyl parahydroxybenzoate are eluted in this order with the resolution being not less than 1.9.

Time span of measurement: About 1.5 times as long as the retention time of thiopental.

Loss on Drying Not more than 2.0 % (1 g, in vacuum, 80 °C, 4 hours).

Assay Weigh accurately about 0.5 g of Thiopental Sodium, previously dried, transfer to a separatory funnel, dissolve in 20 mL of water, add 5 mL of ethanol (95) and 10 mL of dilute hydrochloric acid and extract with 50 mL of chloroform, then with three 25 mL volumes of chloroform. Combine the chloroform extracts, wash with two 5 mL volumes of water and extract the washings with two 10 mL volumes of chloroform. Filter the combined chloroform extracts into an Erlenmeyer flask and wash the filter paper with three 5 mL volumes of chloroform. Combine the filtrate and the washings and add 10 mL of ethanol (95). Titrate with 0.1 mol/L potassium hydroxide-ethanol VS until the color of the solution changes from yellow through pale blue to purple (indicator: 2 mL of alizarin yellow GG-thymolphthalein TS). Perform a blank determination with a mixture of 160 mL of chloroform and 30 mL of ethanol (95) and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 26.43 mg of $C_{11}H_{17}N_2NaO_2S$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Thiopental Sodium for Injection

Thiopental Sodium for Injection is a preparation for injection which is reconstituted before use. Thiopental Sodium for Injection contains not less than 93.0 % and not more than 107.0 % of the labeled amount of thiopental sodium ($C_{11}H_{17}N_2NaO_2S$: 264.32).

Method of Preparation Prepare as directed under Injections, with 100 parts of Thiopental Sodium and 6 parts of Dried Sodium Carbonate by mass.

Description Thiopental Sodium for Injection is a pale yellow powder or mass and has a slight, characteristic odor.
Thiopental Sodium for Injection is very soluble in water and practically insoluble in dehydrated ether.
Thiopental Sodium for Injection is hygroscopic.

Identification (1) Dissolve 0.1 g of Thiopental Sodium for Injection in 10 mL of water and add 0.5 mL of barium chloride TS: a white precipitate is produced. Collect the precipitate and add dilute hydrochloric acid dropwise: the precipitate dissolves with effervescence.

(2) Proceed as directed in the Identification under Thiopental Sodium.

pH Dissolve 1 g of Thiopental Sodium for Injection in 40 mL of water: the pH of this solution is between 10.2 and 11.2.

Purity Proceed as directed in the Purity under Thiopental Sodium.

Loss on Drying Not more than 2.0 % (1 g, in vacuum, 80 °C, 4 hours).

Sterility Test It meets the requirement.

Bacterial Endotoxin Less than 0.3 EU/mg of thiopental sodium.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the require-

ment.

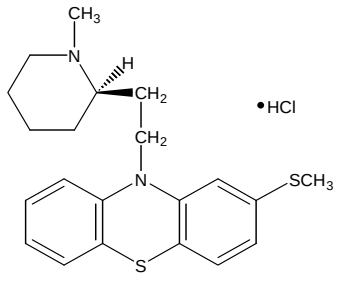
Assay Take 10 samples of Thiopental Sodium for Injection and open each container carefully. Dissolve each content with water, wash each container with water, combine the washings with the former solution and add water to make exactly 1000 mL. Pipet 10 mL of this solution and add water to make exactly 100 mL. Measure exactly a volume (V mL) of this solution, equivalent to about 15 mg of thiopental sodium ($C_{11}H_{17}N_2NaO_2S$) and add water to make exactly 1000 mL. Pipet 10 mL of this solution, add 15 mL of diluted dilute sodium hydroxide TS (1 in 100), add water to make exactly 30 mL and use this solution as the test solution. Separately, weigh accurately about 46 mg of Thiopental RS, previously dried at 105 °C for 3 hours, dissolve in 50 mL of dilute sodium hydroxide TS and add water to make exactly 200 mL. Pipet 2.0 mL of this solution, add water to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry and determine the absorbances, A_T and A_S, at 304 nm, for the test solution and the standard solution, respectively.

Amount (mg) of thiopental sodium ($C_{11}H_{17}N_2NaO_2S$) in each sample of Thiopental Sodium for Injection
= Amount (mg) of Thiopental RS
$$\times \frac{A_T}{A_S} \times \frac{300}{V} \times 1.0907$$

Containers and Storage ***Containers***—Hermetic containers.

Storage—Light-resistant.

Thioridazine Hydrochloride



$C_{21}H_{26}N_2S_2 \cdot HCl$: 407.04

10-[2-(1-Methylpiperidin-2-yl)ethyl]-2-methylsulfanylphenothiazinehydrochloride [*130-61-0*]

Thioridazine Hydrochloride, when dried, contains not less than 99.0 % and not more than 101.0 % of thioridazine hydrochloride ($C_{21}H_{26}N_2S_2 \cdot HCl$).

Description Thioridazine Hydrochloride is a white to pale yellow, crystalline powder, is odorless and has a bitter taste.

Thioridazine Hydrochloride is freely soluble in water, in methanol, in acetic acid (100) or in ethanol (95), sparingly soluble in acetic anhydride and practically insoluble in ether.

Thioridazine Hydrochloride is gradually colored by light.

pH—Dissolve 1.0 g of Thioridazine Hydrochloride in 100 mL of water: the pH of this solution is between 4.2 and 5.2.

Identification (1) Dissolve 10 mg of Thioridazine Hydrochloride in 2 mL of sulfuric acid: a deep blue color is observed.

(2) Dissolve 10 mg of Thioridazine Hydrochloride in 2 mL of water and add 1 drop of ammonium cerium (IV) sulfate TS: a blue color is observed and the color disappears on the addition of excess of the reagent.

(3) Determine the infrared spectra of Thioridazine Hydrochloride and Thioridazine Hydrochloride RS, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(4) To 5 mL of a solution of Thioridazine Hydrochloride (1 in 100), add 2 mL of ammonia TS and heat in a water-bath for 5 minutes. After cooling, filter and acidify the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests (2) for chloride.

Melting Point 159 ~ 164 °C.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Thioridazine Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Selenium***—To 0.1 g of Thioridazine Hydrochloride add 0.1 g of magnesium oxide, mix, transfer to a combustion flask, and proceed as directed under Oxygen Flask Combustion Method, using 25 mL of diluted nitric acid (1 in 30) as the absorbing liquid. Use a 1 L combustion flask. After combustion, wash the stopper and the inside of the flask with 10 mL of water. Transfer the liquid inside the flask to a 150 mL beaker, using about 20 mL of water. Heat gently to boil, boil for 10 minutes, cool to room temperature, and use this solution as the test solution. Separately, pipet 3.0 mL of selenium standard stock solution, add 25 mL of diluted nitric acid (1 in 30) and 25 mL of water, and use this solution as the standard solution. Adjust the pH of the test solution and standard solution to 2.0 ± 0.2 with diluted ammonia solution (28) (1 in 2), add water to make exactly 60 mL, transfer to a separatory funnel using 10 mL of water, and wash the separatory funnel with 10 mL of water. Add 0.2 g of hydroxylamine hydrochloride, stir to dissolve, add immediately 5.0 mL of 2,3-diaminonaphthalene TS, stopper, stir to mix, and allow to stand at room temperature for 100 minutes. Add 5.0 mL of cyclohexane, shake vigorously for 2 minutes, allow the layers to separate, discard the water

layer, centrifuge the cyclohexane extract to remove water, and take the cyclohexane layer. Determine the absorbances at about 380 nm of these solutions as directed under Ultraviolet-visible Spectrophotometry, using a solution prepared by adding 25 mL of water to 25 mL of diluted nitric acid (1 in 30) and proceeding in the same manner, as the blank: the absorbance obtained from the test solution is not more than that from the standard solution (not more than 30 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Thioridazine Hydrochloride according to Method 3 and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 0.10 g of Thioridazine Hydrochloride in 10 mL of methanol and use this solution as the test solution. Pipet 1 mL of the test solution, add methanol to make exactly 20 mL, pipet 2 mL of this solution, add methanol to make exactly 10 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. After air-drying, immediately develop the plate with a mixture of chloroform, 2-propanol and ammonia solution (28) (74 : 25 : 1) to a distance of about 10 cm and air-dry the plate. After cooling, examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and the spots of the starting point from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

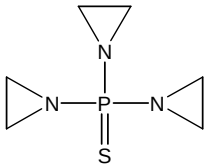
Assay Weigh accurately about 0.35 g of Thioridazine Hydrochloride, previously dried, dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (1 : 1) and titrate with 0.1 mol/L perchloric acid (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid
= 40.70 mg $C_{21}H_{26}N_2S_2 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Thiotepa



$C_6H_{12}N_3PS$: 189.22

tris(Aziridin-1-yl)-sulfanylidene-λ5-phosphane
[*52-24-4*]

Thiotepa, when dried, contains not less than 98.0 % and not more than 101.0 % of thiotepa ($C_6H_{12}N_3PS$).

Description Thiotepa appears as colorless or white crystals or crystalline powder and is odorless.
Thiotepa is freely soluble in water, in ethanol (95) or in ether.
Dissolve 1.0 g of Thiotepa in 10 mL of water: the solution is neutral.

Identification (1) To 5 mL of a solution of Thiotepa (1 in 100), add 1 mL of ammonium molybdate TS and allow to stand: a dark blue color develops slowly when the solution is cold, or quickly when warm.

(2) To 5 mL of a solution of Thiotepa (1 in 100), add 1 mL of nitric acid: this solution responds to the Qualitative Tests (2) for phosphate.

(3) Dissolve 0.1 g of Thiotepa in a mixture of 1 mL of lead acetate TS and 10 mL of sodium hydroxide TS and boil: the gas evolved changes moistened red litmus paper to blue and the solution shows a grayish red color.

Melting Point 52 ~ 57 °C.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Thiotepa in 20 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 1.0 g of Thiotepa in a platinum crucible according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Dissolve 0.20g of Thiotepa in 5 mL of water and add 1 mL of nitric acid and 1 mL of sulfuric acid. Prepare the test solution with this solution according to Method 2 and perform the test (not more than 10 ppm).

Loss on Drying Not more than 0.2 % (1 g, in vacuum, silica gel, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g, platinum crucible).

Assay Weigh accurately about 0.1 g of Thiotepa, previously dried, dissolve in 50 mL of a solution of potassium thiocyanate (3 in 20), add 25 mL of 0.05

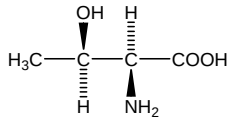
mol/L sulfuric acid VS, exactly measured and allow to stand for 20 minutes with occasional shaking. Titrate the excess sulfuric acid with 0.1 mol/L sodium hydroxide VS. The end point titration is only when the color of the solution changes from red to pale yellow (indicator: 3 drops of methyl red TS). Perform a blank determination and make any necessary correction.

Each mL of 0.05 mol/L sulfuric acid VS
= 6.307 mg of $C_6H_{12}N_3PS$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and in a cold place.

L-Threonine



$C_4H_9NO_3$: 119.12

(2*S*,3*R*)-2-Amino-3-hydroxybutanoic acid [*72-19-5*]

L-Threonine, when dried, contains not less than 98.5 % and not more than 101.0 % of L-threonine ($C_4H_9NO_3$).

Description L-Threonine appears as white crystals or crystalline powder, is odorless or has a slight, characteristic odor and has a slightly sweet taste.
L-Threonine is freely soluble in formic acid, soluble in water, and practically insoluble in ethanol (95) or in ether.

Identificatlon Determine the infrared spectra of L-Threonine and L-Threonine RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: -26.0 ~ -29.0° (after drying, 1.5 g, water, 25 mL, 100 mm).

pH Dissolve 0.20 g of L-Threonine in 20 mL of waters: the pH of this solution is between 5.2 and 6.2.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of L-Threonine in 20 mL of water: the solution is clear and colorless.

(2) ***Chloride***—Perform the test with 0.5 g of L-Threonine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021 %).

(3) ***Sulfate***—Perform the test with 0.6 g of L-Threonine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028 %).

(4) ***Ammonium***—Perform the test with 0.25g of L-Threonine. Prepare the control solution with 5.0 mL of standard ammonium solution (not more than 0.02 %).

(5) ***Heavy metals***—Proceed with 1.0 g of L-Threonine according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(6) ***Iron***—Dissolve 0.333 g of L-Threonine in water to make 45 mL, add 2 mL of hydrochloric acid, and use this solution as the test solution. To 1.0 mL of standard iron solution add water to make 45 mL, add 2 mL of hydrochloric acid, and use this solution as the standard solution. To the test solution and standard solution add 50 mg of ammonium peroxydisulfate and 3 mL of 30 % ammonium thiocyanide solution, and mix: the color of the test solution is not more intense than that of the standard solution (not more than 30 ppm).

(7) ***Arsenic***—Dissolve 1.0 g of L-Threonine in 5 mL of dilute hydrochloric acid and perform the test with this solution as the test solution (not more than 2 ppm).

(8) ***Related substances***—Dissolve 0.30 g of L-Threonine in water to make exactly 50 mL and use this solution as the test solution. Pipet 1 mL of this solution, add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot each 5 μL of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, acetic acid (100) (3 : 1 : 1) to a distance of about 10 cm and air-dry the plate at 80 °C for 30 minutes, Spray evenly a solution of ninhydrin in acetone (1 in 50) upon the plate and dry at 80 °C for 5 minutes: the spots other than the principal spot are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.2 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.12 g of L-Threonine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.01 mol/L perchloric acid VS
= 11.912 mg of $C_4H_9NO_3$

Containers and Storage ***Containers***—Tight containers.

Thrombin

Thrombin is prepared from prothrombin obtained from blood of man or bull, through interaction with added thromboplastin in the presence of calcium ions, sterilized and lyophilized.
Thrombin contains not less than 80.0 % and not more than 150.0 % of the labeled units of thrombin.
Each mg of Thrombine contains not less than 10 units of thrombin.

Description Thrombin is a white to pale yellow, amorphous substance.
Thrombin equivalent to 500 units is dissolved in 1.0 mL of Isotonic Sodium Chloride Injection: this solution is clear or slight turbid within 1 minute.

Loss on Drying Not more than 3.0 % (50 mg, in vacuum, P_2O_5, 4 hours).

Sterility Test It meets the requirement.

Assay (1) ***Fibrinogen solution***—Weigh accurately about 30 mg of fibrinogen and dissolve in 3 mL of Isotonic Sodium Chloride Injection. Allow the solution to clot sufficiently with frequent shaking after the addition of about 3 units of Thrombin. Wash the precipitated clot thoroughly until the washings yield no turbidity on addition of silver nitrate TS, weigh the clot after drying at 105 °C for 3 hours and calculate the content of the clot in the fibrinogen. Dissolve the fibrinogen in Isotonic Sodium Chloride Injection so that the clot should be 0.20 %, adjust the pH of the solution between 7.0 and 7.4 by addition of 0.05 mol/L sodium hydrogen phosphate TS (or if necessary, use 0.5 mol/L of sodium hydrogen phosphate TS) and dilute with Isotonic Sodium Chloride Injection to make a 0.1 % solution.

(2) ***Procedure***—Dissolve Thrombin RS in Isotonic Sodium Chloride Injection and prepare four standard solutions which contain 4.0, 5.0, 6.2 and 7.5 units in 1 mL, respectively. Transfer accurately 0.10 mL each of the standard solutions maintained at a given temperature ± 1 °C between 20 °C and 30 °C to a small test tube, 10 mm in internal diameter, 100 mm in length, blow out 0.90 mL of the fibrinogen solution at the same temperature into the test tube from a pipet, start a stop-watch simultaneously, shake the tube constantly and determine the time for the first appearance of clot. Calculate the average values of five determinations for the four standard solutions, respectively. If the deviation between the maximum and the minimum values of five determinations is more than 10 % of the average value, reject the whole run and try the experiment again. The concentration of the standard solution may be changed appropriately within the range between 14 and 60 seconds of the clotting time. The determination proceeds at the same temperature described above. Next, weigh accurately the whole contents of a single container of Thrombin, dissolve it in Isotonic Sodium Chloride Injection to provide a solution which is presumed to contain about 5 units in each mL, treat 0.10 mL of the solution with the same reagents in the same manner 5 times, determine the clotting times and calculate the average value. Plot the average values of the clotting times of the four standard solutions on a logarithmic graph, using units as the abscissa and clotting times as the ordinate and draw a calibration line which best fits the four plotted points. Using this line, read the units (U) from the average value of the clotting times of the test solution.

$$\text{Units of a single container of Thrombin} = U \times 10 \times V$$

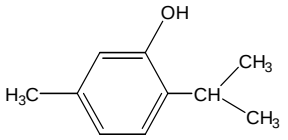
V: Number of mL of the volume in which the contents of a single container of Thrombin has been dissolved.

Calculate the units per 1 mg of the contents.

Containers and Storage ***Containers***—Hermetic containers.

Storage—Not exceeding 10 °C.

Thymol



$C_{10}H_{14}O$: 150.22

5-Methyl-2-propan-2-ylphenol [*89-83-8*]

Thymol contains not less than 98.0 % and not more than 101.0 % of thymol ($C_{10}H_{14}O$).

Description Thymol appears as colorless crystals or white, crystalline masses, has an aromatic odor and has a burning taste.
Thymol is very soluble in acetic acid (100), freely soluble in ethanol (95) or in ether, and slightly soluble in water.
Thymol sinks in water, but when warmed, it melts and rises to the surface of water.

Identification (1) To 1 mL of a solution of Thymol in acetic acid (100) (1 in 300), add 6 drops of sulfuric acid and 1 drop of nitric acid: a blue-green color is observed by reflected light and a red-purple color is observed by transmitted light.

(2) Dissolve 1 g of Thymol in 5 mL of solution of sodium hydroxide (1 in 10) by heating in a water-bath and continue heating for several minutes: a pale yellow-red color is slowly observed. Allow this solution to stand at room temperature: the color changes to dark

yellow-brown. Shake this solution with 2 to 3 drops of chloroform: a purple color is gradually observed.

(3) Triturate Thymol with an equal mass of camphor or menthol: the mixture liquefies.

Melting Point 49 ~ 51 °C.

Purity (1) ***Non-volatile residue***—Volatilize 2.0 g of Thymol by heating on a water-bath and dry the residue at 105 °C for 2 hours: the mass is not more than 1.0 mg.

(2) ***Other phenols***—Shake vigorously 1.0 g of Thymol with 20 mL of warm water for 1 minute and filter. To 5 mL of the filtrate, add 1 drop of iron (III) chloride TS: a green color is observed, but no blue to purple color is observed.

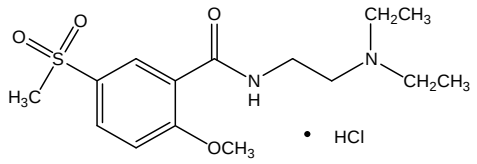
Assay Weigh accurately about 0.5 g of Thymol, dissolve in 10 mL of sodium hydroxide TS and add water to make exactly 100 mL. Measure exactly 10 mL of the solution into an iodine flask, add 50 mL of water and 20 mL of dilute sulfuric acid and cool in ice water for 30 minutes. Add exactly 20 mL of 0.05 mol/L bromine VS, stopper tightly immediately, allow to stand for 30 minutes in ice-water with occasional shaking in a dark place, add 14 mL of potassium iodide TS and 5 mL of chloroform, stopper tightly, shake vigorously and titrate the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Stopper tightly, shake vigorously near the end point and continue the titration until the blue color in the chloroform layer disappears. Perform a blank determination and make any necessary correcting.

Each mL of 0.05 mol/L bromine VS
= 3.756 mg of $C_{10}H_{14}O$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Tiapride Hydrochloride



$C_{15}H_{24}N_2O_4S \cdot HCl$: 364.89

N-[2-(Diethylamino)ethyl]-2-methoxy-5-methylsulfonylbenzamidehydrochloride [*51012-33-0*]

Tiapride Hydrochloride contains not less than 98.5 % and not more than 101.0 % of tiapride hydrochloride ($C_{15}H_{24}N_2O_4S \cdot HCl$), calculated on the dried basis.

Description Tiapride Hydrochloride appears as white to pale yellowish white crystalline powder.
Tiapride Hydrochloride is very soluble in water, soluble in methanol, and slightly soluble in ethanol (95).

Identification (1) Determine the infrared spectra of Tiapride Hydrochloride and Tiapride Hydrochloride RS, as directed in the potassium bromide disk method under Infrared Spectrophotornetry : both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) A solution of Tiapride Hydrochloride (2.5 in 50) responds to the Qualitative Tests for chloride.

pH The pH of a solution obtained by dissolving 2.5 g of Tiapride Hydrochloride in 50 mL of water is between 4.0 and 6.0.

Purity (1) ***Clarity and color of solution***—A solution of 2.5 g of Tiapride Hydrochloride in 50 mL of water is clear. When the test is performed with this solution as directed under Ultraviolet-visible Spectrophotometry, the absorbance at the wavelength of 450 nm is not more than 0.030.

(2) ***Heavy metals***—Proceed with 1.0 g of Tiapride Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) ***Related substance I***—Dissolve 0.40 g of Tiapride Hydrochloride, accurately weighed, in methanol to make exactly 10 mL and use this solution as the test solution. Seperately, dissolve 20.0 mg of tiapride related substance I RS {*N,N*-diethylethane-1,2-diamine} in methanol to make exactly 50 mL. To 2.0 mL of this solution, add methanol to make exactly 20 mL and use this solution as the standard solution. Perform the test with these solutions, as directed under Thin Layer Chromatography. Spot 10 μL each of the test solution and the standard solution on silica gel plate for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, methanol, 1,4-dioxane and ammonia solution (28) (90 : 14 : 10 : 2) to a distance of about 12 cm, and air-dry the plate. Spray with 0.2 w/v % ninhydrin 1-butanol solution and heat at 100 °C for 15 minutes: the spot of the related substance I from the test solution is not more intense than the spot from the standard solution (not more than 0.1 %).

(4) ***Related substances***—Dissolve 0.1 g of Tiapride Hydrochloride, accurately weighed, in the mobile phase to make exactly 100 mL and use this solution as the test solution. To 1.0 mL of the test solution, add the mobile phase to make exactly 10 mL. To 1.0 mL of this solution, add the mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution, as directed under Liquid Chromatography, according to the following operating conditions and measure the areas of peaks: the area of any peak, other than the principal peak, from the test solution is not more than the area of the principal peak from the standard solution (0.1 %), and the sum of the areas of

any such peaks is not more than 3 times the area of the principal peak from the standard solution (0.3 %). Disregard any peak with an area not more than 0.5 times the area of the principal peak from the standard solution (not more than 0.05 %)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: Dissolve 5.44 g of potassium dihydrogen phosphate and 80 mg of sodium ocatanesulphonate in 780 mL of water, adjust the pH to 2.7 with phosphoric acid and add water to make 800 mL. Add 150 mL of methanol and 50 mL of acetonitrile to this solution.

Flow rate: 1.5 mL/minute.

System suitability

System performance: Dissolve 5.0 mg of Tiapride Hydrochloride RS and 5.0 mg of tiapride *N*-oxide RS in the mobile phase to make 100 mL. When the procedure is run with 10 μL each of this solution under the above operating conditions, the resolution between the peaks of tiapride (the retention time is about 9 minutes) and tiapride *N*-oxide (the retention time is about 13 minutes) is not less than 4.0.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, constant mass).

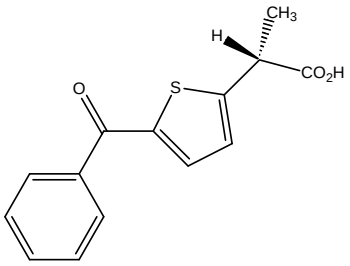
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.3 g of Tiapride Hydrochloride, dissolve in 20 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in the Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 36.489 mg of $C_{15}H_{24}N_2O_4S \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Tiaprofenic Acid



and enantiomer

$C_{14}H_{12}O_3S$: 260.31

2-(5-Benzoylthiophen-2-yl)propanoic acid [*33005-95-7*]

Tiaprofenic Acid contains not less than 99.0 % and not more than 101.0 % of tiaprofenic acid ($C_{14}H_{12}O_3S$), calculated on the dried basis.

Description Tiaprofenic Acid is a white, crystalline powder.

Tiaprofenic Acid is very soluble in acetone, in ethanol (95) or in dichloromethane, and practically insoluble in water.

Identification (1) Dissolve each 25.0 mg of Tiaprofenic Acid and Tiaprofenic Acid RS in 0.01 mol/L ethanolic hydrochloric acid TS to 50.0 mL. Add 0.01 mol/L ethanolic hydrochloric acid TS to 1.0 mL of this solution to make 50 mL. Determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Tiaprofenic Acid and Tiaprofenic Acid RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Dissolve 10 mg of Tiaprofenic Acid in dichloromethane to make 10 mL, and use this solution as the test solution. Separately, dissolve 10 mg of Tiaprofenic Acid RS in dichloromethane to make 10 mL, and use this solution as the standard solution (1). Dissolve 10 mg of Ketoprofene RS in dichloromethane to make 10 mL, add the standard solution (1) to 1 mL of this solution, and use this solution as the standard solution (2). Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL of each solution on a plate of silica gel with a fluoroscent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone, dichloromethane and acetic acid (31) (80 : 20 : 1) to a distance of about 15 cm, and air-dry the plate examine under ultraviolet light (main wavelanght : 254 nm). The principal spot obtained from the test solution and the principal spot obtained from the standard solution (1) show the same R_f value. This test is effective when two principal spots are clearly separated in the chromatogram

of the standard solution (2).

Melting Point 95 ~ 99 °C.

Specific Optical Rotation $[\alpha]_D^{20}$: -0.10 ~ +0.10 (0.5 g, ethylacetate,10 mL, 100 mm)

Purity (1) ***Clarity and color of solution***—Dissolve about 2.0 g of Tiaprofenic Acid in ethanol (95) to make 20 mL. This solution is clear and not more than intensely colored than a mixture of 5 mL of control solution and 95 mL of 1 w/v hydrochloric acid.

(2) ***Heavy metals***—Proceed with 2.0 g of Tiaprofenic Acid according to method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Dissolve 20.0 mg of Tiaprofenic Acid in the mobile phase to make 20 mL, and use this solution as the test solution. Add the mobile phase to 1.0 mL of test solution to make exactly 50 mL, add the mobile phase to 1.0 mL of this solution to make exactly 10 mL, and use this solution as the standard solution (1). Add the mobile phase to 5.0 mL of the standard solution (1) to make exactly 10 mL, and use this solution as the standard solution (2). Dissolve 10.0 mg of Tiaprofenic Acid related substance I RS [(2RS)-2-(5-benzoyl-thiopene-3-yl)propanosan] in the mobile phase to make 100 mL. Add the mobile phase to 1.0 mL of this solution to make exactly 50 mL, and use this solution as the standard solution (3). Add the standard solution (3) to 1.0 mL of the standard solution (1) to make exactly 2 mL, and use this solution as the standard solution (4). Perform the test with each 20 μL of the test solution and the standard solutions as directed under Liquid Chromatography according to the following operating conditions. The peak area of the related substance I from the test solution is not greater than the peak area of that from the standard solution (3) (not more than 0.2 %). The peak area, other than the principal peak and the peak area of the related substance I are not greater than the area of the principal peak obtained from the standard solution (2) (0.1 %), the sum of the areas of all the peaks, other than the principal peak and the peak of the related substance I, is not greater than 1.5 times the area of the principal peak obtained from the standard solution (1) (0.3 %). Disregard any peak with an area less than 0.5 times the area of the principal peak obtained from the standard solution (2).

Operating conditions

Detector : An ultraviolet absorption photometer (wavelength: 250 nm)

Column : A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Mobile phase : A mixture of dichloromethane, hexane, acetic acid (100) and water (500 : 500 : 20 : 1). Add the water to acetic acid (100), then hexane and dichloromethane. Sonicate the mixture for 2 minutes. Do not degas with helium during analysis.

Flow rate : 1.0 mL/minute

System suitability

System performance : when the procedure is run with 20 μL each of the test solution and the standard solution (4), as directed under the above operating conditions, the relative retention time of related substance II, III and I are 0.19, 0.48 and 0.86 to the retention time of tiaprofenic acid, respectively, with the resolution of the peak between tiaprofenic acid and the peak of related substance I being not less than 3.0. Adjust the sensitivity of the system so that the heights of the main peak obtained from the standard solution (4) is not less than 50 % of the full scale of the recorder.

Loss on Drying Not more than 0.5 % (1.0 g, 60 °C, not more than 0.9 kPa, 3 hours).

Residue on Ignition Not more than 0.1 % (1.0 g).

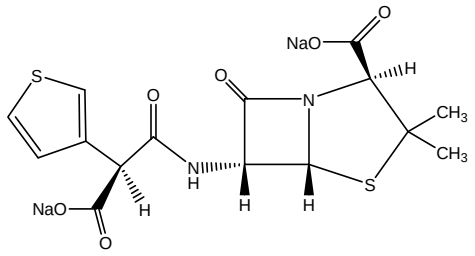
Assay Weigh accurately about 0.25 g of Tiaprofenic Acid, dissolve in 25 mL of ethanol (95), add 25 mL of water, and titrate with 0.1 mol/L sodium hydroxide (Indicator: 0.5 mL of phenolphthalein TS). Perform a blank determination and make any necessary correction.

1 mL of 0.1 mol/L sodium hydroxide
= 26.03 mg of $C_{14}H_{12}O_3S$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Ticarcillin Sodium



$C_{15}H_{14}N_2Na_2O_6S_2$: 428.39

Disodium(2*S*,5R,6*R*)-6-[[(2*R*)-2-carboxylato-2-thiophen-3-ylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [*4697-14-7*]

Ticarcillin Sodium contains not less than 800 μg (potency) per mg of ticarcillin ($C_{15}H_{16}N_2O_6S_2$: 384.43), calculated on the anhydrous basis.

Description Ticarcillin Sodium is a white to pale yellowish white powder, and has not a characteristic odor.

Ticarcillin Sodium is very soluble in water, freely soluble in methanol, sparingly soluble in ethanol (95), and practically insoluble in ether.

Identification (1) Weigh accurately about 40 mg each of Ticarcillin Sodium and Ticarcillin Sodium RS, dissolve in water to make exactly 100 mL, pipet 5 mL each of these solutions and add 0.1 mol/L methanolic hydrochloric acid TS to make exactly 100 mL, and use these solutions as the test solution and the standard solution. Determine the absorption spectra of these solutions between 200 nm and 300 nm, using 0.1 mol/L methanolic hydrochloric acid TS as the blank, as directed under Ultraviolet-visible Spectrophotometry, they exhibit maximum about 230 nm and similar intensities of absorption at the same wavelengths.

(2) A solution of Ticarcillin Sodium (1 in 20) responds to the Qualitative Tests for sodium.

Specific Optical Rotation $[\alpha]_D^{25}$: +172 ~ +187° (1 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.1 g of Ticarcillin Sodium in 10 mL of water is between 6.0 and 8.0.

Purity (1) ***Dimethylaniline***—Weigh accurately about 1.0 g of Ticarcillin Sodium, add 5 mL of 1 mol/L sodium hydroxide TS and 1 mL of the internal standard solution, centrifuge if necessary, and use the clear supernatant liquid as the test solution. Separately, weigh accurately about 50 mg of dimethylaniline, and add 2.0 mL of hydrochloric acid and water to make 50 mL. Pipet 5.0 mL of this solution, and add water to make exactly 250 mL. Pipet 1.0 mL of this solution, add 5.0 mL of 1 mol/L sodium hydroxide TS and 1.0 mL of the internal standard solution, centrifuge if necessary, and use the clear supernatant liquid as the standard solution. Perform the test with 1 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of dimethylaniline to that of the internal standard in the test solution and standard solution (not more than 20 ppm).

$$\text{Content (ppm) of dimethylaniline} = \frac{Q_T}{Q_S} \times \frac{\text{Amount (mg) of dimethylaniline taken}}{\text{Amount (mg) of Ticarcillin Sodium taken}} \times 4$$

Internal standard solution—Dissolve 50.0 mg of naphthalene in cyclohexane to make 50 mL. To 5.0 mL of this solution add cyclohexane to make 100 mL, and use this solution as the internal standard solution.

Operating conditions

Detector: A hydrogen flame ionization detector

Column: A column, about 2 mm in internal diameter and 2 m in length, packed with diatomaceous earth for gas chromatography, coated with 50 % phenyl-50 % methylpolysiloxane for gas chromatography at the rate of 3 %.

Column temperature: 120 °C

Injection port and detector temperature: 150 °C

Carrier gas: Nitrogen

Flow rate: 30 mL/minute

(2) ***Ticarcillin content***—Weigh accurately about 40 mg each of Ticarcillin Sodium and Ticarcillin Sodium RS, and dissolve in water to make exactly 100 mL. Pipet 5.0 mL of each solution, add 0.1 mol/L methanolic hydrochloric acid TS to make exactly 100 mL, and use these solutions as the test solution and standard solution, respectively. Determine the absorbances, A_T and A_S, at 230 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry, using the blank solution as the blank (80.0 to 94.0 %, calculated on the anhydrous basis).

$$\text{Content (\%) of ticarcillin} = \text{Concentration (\%) of ticarcillin in Ticarcillin Sodium RS} \times \frac{\text{Amount (mg) of Ticarcillin Sodium RS}}{\text{Amount (mg) of the sample}} \times \frac{A_T}{A_S}$$

0.1 mol/L Hydrochloric acid-methanol TS—To 0.8 mL of hydrochloric acid add methanol to make 100 mL.

Water Not more than 6.0 % (0.2 g, volumetric titration, direct titration).

Sterility Test It meets the requirement, when Ticarcillin Sodium is used in a sterile preparation.

Bacterial Endotoxins Less than 0.05 EU/mg (potency) of ticarcillin, when Ticarcillin Sodium is used in a sterile preparation.

Assay Weigh accurately about 50 mg (potency) each of Ticarcillin Sodium and Ticarcillin Sodium RS, dissolve each in phosphate buffer solution (pH 6.4) to make exactly 50 mL, and use these solutions as the test solution and standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of the test solution and standard solution.

$$\text{Amount [μg (potency)] of ticarcillin } (C_{15}H_{16}N_2O_6S_2) = \text{Amount [μg (potency)] of Ticarcillin RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 30 cm in length, packed

with octadecylsilanized silica gel for liquid chromatography (3 to 10 μm in particle diameter).

Mobile phase: A mixture of phosphate buffer solution (pH 4.3) and acetonitrile (95 : 5)

Flow rate: 2 mL/minute

System suitability

System performance: When the procedure is run with the resolution solution under the above operating conditions, the relative retention times of clavulanic acid and ticarcillin are about 0.2 and 1.0, respectively, the resolution is not less than 5.0, and the number of theoretical plates and symmetry factor are not less than 1000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 5 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ticarcillin is not more than 2.0 %.

Phosphate buffer solution (pH 6.4)—Dissolve 6.9 g of sodium dihydrogen phosphate monohydrate in 900 mL of water, adjust the pH to 6.4 ± 0.1 with 10 mol/L sodium hydroxide, and add water to make 1000 mL.

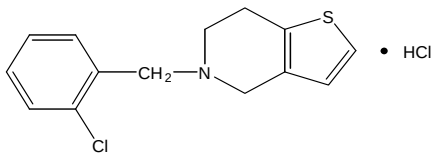
Phosphate buffer solution (pH 4.3)—Dissolve 13.8 g of sodium dihydrogen phosphate monohydrate in 900 mL of water, adjust the pH to 4.3 ± 0.1 with 10 mol/L sodium hydroxide, and add water to make 1000 mL.

Resolution solution—Weigh accurately about 25 mg (potency) of Ticarcillin Sodium RS, add 5 mL of a solution of Clavulanic Acid RS in phosphate buffer solution (pH 6.4) containing 0.15 mg per mL, and add phosphate buffer solution (pH 6.4) to make exactly 25 mL.

Containers and Storage ***Containers***—Tight containers.

Storage—At a temperature between 2 to 8 °C.

Ticlopidine Hydrochloride



$C_{14}H_{14}ClNS \cdot HCl$: 300.25

5-[(2-Chlorophenyl)methyl]-4*H*,5*H*,6*H*,7*H*-thieno[3,2-c]pyridine hydrochloride [*53885-35-1*]

Ticlopidine Hydrochloride contains not less than 99.0 % and not more than 101.0 % of ticlopidine hydrochloride ($C_{14}H_{14}ClNS \cdot HCl$), calculated on the anhydrous basis.

Description Ticlopidine Hydrochloride is a white to pale yellowish white crystalline powder.

Ticlopidine Hydrochloride is freely soluble in acetic acid (100), soluble in water or in methanol, sparingly soluble in ethanol (95) and practically insoluble in ether.

Identification (1) Determine the infrared spectra of Ticlopidine Hydrochloride and Ticlopidine Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) A solution of Ticlopidine Hydrochloride (1 in 20) responds to the Qualitative Tests (2) for chloride.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Ticlopidine Hydrochloride according to Method 3 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Arsenic***—Prepare the test solution with 1.0 g of Ticlopidine Hydrochloride according to Method 4 and perform the test (not more than 2 ppm).

(3) ***Related substances***—Dissolve 0.5 g of Ticlopidine Hydrochloride in 20 mL of solution of hydrochloric acid in methanol (1 in 20000) and use this solution as the test solution. Pipet 5 mL of the test solution, add a solution of hydrochloric acid in methanol (1 in 20000) to make exactly 200 mL and use this solution as the standard solution (1). Separately, pipet 1 mL of the test solution, add a solution of hydrochloric acid in methanol (1 in 20000) to make exactly 50 mL and use this solution as the standard solution (2). Perform the test with the test solution and standard solutions (1) and (2) as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution (1) on a plate of silica gel for thin-layer chromatography (plate 1) and spot 10 μL each of the test solution and the standard solution (2) on another plate of silica gel with a fluorescence indicator for thin-layer chromatography (plate 2). Develop the plates with an upper layer of a mixture of water, 1-butanol and acetic acid (100) (5 : 4 : 1) to a distance of about 15 cm and air-dry the plates. Spray evenly a solution of ninhydrin in acetone (1 in 50) on plate 1 and heat at 100 °C for 20 minutes: the spots other than principal spot from the test solution are not more intense than the spot from the standard solution (1). Allow plate 2 to stand in iodine vapor for 30 minutes: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution (2).

(4) ***Formaldehyde***—Dissolve 0.80 g of Ticlopidine Hydrochloride in 19.0 mL of water, add 1.0 mL of 4 mol/L sodium hydroxide TS, shake well. Centrifuge and filter the supernatant liquid. Pipet 5.0 mL of the filtrate, add 5.0 mL of acetylacetone TS, mix and warm at 40 °C for 40 minutes: the solution has no more color than the following control solution.

Control solution—Weigh accurately 0.54 g of formaldehyde solution and add water to make exactly 1000 mL. Pipet 10.0 mL of this solution, add water to make exactly 1000 mL. Prepare before use. To 8.0 mL of this solution, add water to make 20.0 mL and filter. To 5.0 mL of the filtrate, add 5.0 mL of acetylacetone TS and proceed in the same manner.

Water Not more than 1.0 % (0.3 g, volumetric titration, direct titration).

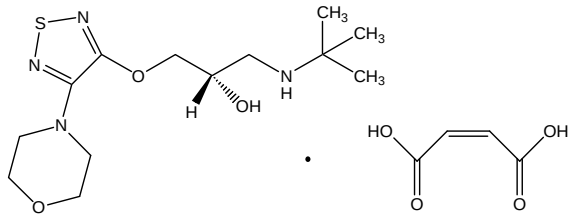
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.4 g of Ticlopidine Hydrochloride, dissolve in 20 mL of acetic acid (100), add 40 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 30.025 mg of $C_{14}H_{14}ClNS \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Timolol Maleate



$C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$: 432.49

(*Z*)-But-2-Enedioic acid; (2*S*)-1-(*te*rt-butylamino)-3-[(4-morpholin-4-yl-1,2,5-thiadiazol-3-yl)oxy]propan-2-ol [*26921-17-5*]

Timolol Maleate, when dried, contains not less than 98.0 % and not more than 101.0 % of timolol maleate ($C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$).

Description Timolol Maleate is a white to pale yellowish white crystalline power.
Timolol Maleate is freely soluble in acetic acid (100), and soluble in water or in ethanol (99.5).
Timolol Maleate dissolves in 0.1 mol/L hydrochloric acid TS.

Melting point—about 197 °C (with decomposition).

Identification (1) Determine the absorption spectra of a solution of Timolol Maleate and Timolol Maleate RS in 0.1 mol/L hydrochloric acid TS (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Timolol Maleate and Timolol Maleate RS as directed in the potassium bromide disk method under Infrared Spectrophotornetry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) To 5 mL of a solution of Timolol Maleate (1 in 500) add 1 drop of potassium permanganate TS: the red color of the TS disappears immediately.

Specific Optical Rotation $[\alpha]_D^{20}$: -5.7 ~ -6.5° (after drying, 1.25 g, 1 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH The pH of a solution prepared by dissolving 1.0 g of Timnolol Maleate in 20 mL of water is between 3.8 and 4.3.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Timolol Maleate in 20 mL of water: the solution is clear, and its absorbance at 440 nm, determined as directed under Ultraviolet-visible Spectrophotometry is not more than 0.05.

(2) ***Heavy metals***—Proceed with 2.0 g of Timolol Maleate according to Method 4, arid perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) ***Related substances***—Dissolve 30 mg of Timolol Maleate in 20 mL of the mobile phase and use this solution as the test solution. Pipet 1 mL of the test solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 25 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than timolol and maleic acid is not larger than 0.2 times the peak area of timolol from the standard solution, and the total area of the peaks other than the peak of timolol and maleic acid is not larger than 0.5 times the peak area of timolol from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in internal diameter and 25 cm in length, packed with phenylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: Dissolve 1.9 g of sodium 1-hexanesulfonate in 1800 mL of water, add 6.0 mL of triethylamine and 8.0 mL of formic acid, adiust to pH 3.0 with formic acid, and add water to make 2000 mL. To 1400 mL of this solution add 500 mL of methanol and 100 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of timolol is about 18 minutes.

System suitability

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make 10 mL. Confirm that the peak area of timolol obtained from 25 μL of this solution is equivalent to 7 % to 13 % of that from 25 μL of the standard solution.

System performance: When the procedure is run with 25 μL of the test solution under the above operating condition, the number of theoretical plates and the symmetry factor of the peak of timolol are not less than 1500 and not more than 2.5, respectively.

System repeatability: When the test is repeated 6 times with 25 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of timolol is not more than 2.0 %.

Time span of measurement: About 2 times as long as the retention time of timolol beginning after the solvent peak.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, 100 °C, 3 hours).

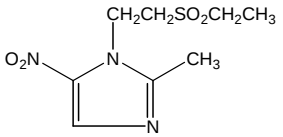
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.8 g of Timolol Maleate, previously dried, dissolve in 90 mL of acetic acid (100), and titrate with 0.1 mol/L perchioric acid VS (potentiometric titration, Endpoint Detection method in Titrimetry). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 43.25 mg of $C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$

Containers and Storage ***Containers***—Tight containers.

Tinidazole



$C_8H_{13}N_3O_4S$: 247.27

1-(2-Ethylsulfonylethyl)-2-methyl-5-nitroimidazole
[*19387-91-8*]

Tinidazole, when dried, contains not less than 98.5 % and not more than 101.0 % of tinidazole ($C_8H_{13}N_3O_4S$).

Description Tinidazole is a pale yellow, crystalline powder.
Tinidazole is soluble in acetic anhydride or in acetone, sparingly soluble in methanol or in ethanol (95), and very slightly soluble in water or in ether.

Identification (1) Determine the absorption spectra of solutions of Tinidazole and Tinidazole RS in methanol (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Tinidazole and Tinidazole RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 125 ~ 129 °C.

Purity (1) ***Sulfate***—To 2.0 g of Tinidazole, add 100 mL of water, boil for 5 minutes, cool, add water to make 100 mL and filter. Take 25 mL of the filtrate and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Use this solution as the test solution and perform the test. Prepare the control solution with 0.45 mL of 0.005 mol/L sulfuric acid VS (not more than 0.043 %).

(2) ***Heavy metals***—Proceed with 1.0 g of Tinidazole according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Arsenic***—Prepare the test solution with 2.0 g of Tinidazole according to Method 3 and perform the test (not more than 1 ppm).

(4) ***Related substances***—Dissolve 50 mg of Tinidazole in 20 mL of acetone and use this solution as the test solution. Pipet 1 mL of the test solution, add acetone to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and diethylamine (19 : 1) to a distance of about 10 cm, air-dry the plate, heat at 100 °C for 5 minute and cool. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 1.0 % (1 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

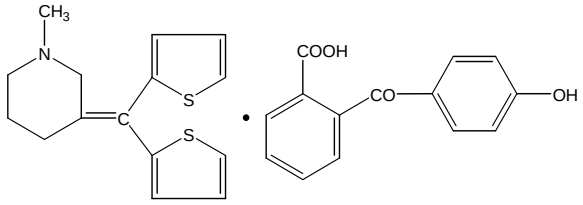
Assay Weigh accurately about 0.35 g of Tinidazole, previously dried, dissolve in 50 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 24.727 mg of $C_8H_{13}N_3O_4S$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Tipepidine Hibenzate



$C_{15}H_{17}NS_2C_{14}H_{10}O_4$: 517.66

4-(2-Carboxybenzoyl)phenolate;3-(dithiophen-2-ylmethylidene)-1-methylpiperidin-1-ium [*31139-87-4*]

Tipepidine Hibenzate, when dried, contains not less than 98.5 % and not more than 101.0 % of tipepidine hibenzate ($C_{15}H_{17}NS_2C_{14}H_{10}O_4$).

Description Tipepidine Hibenzate is a white to pale yellow, crystalline powder, is odorless and tasteless.
Tipepidine Hibenzate is freely soluble in acetic acid (100), slightly soluble in methanol or in ethanol (95), very slightly soluble in water and practically insoluble in ether.

Identification (1) Dissolve 10 mg of Tipepidine Hibentate in 5 mL of sulfuric acid: an orange color is observed.

(2) Dissolve 0.3 g of Tipepidine Hibenzate in 10 mL of sodium hydroxide TS and 5 mL of water and extract with two 20 mL volumes of chloroform. Wash the chloroform extracts with 10 mL of water and filter the chloroform layer. Evaporate the filtrate on a water-bath to dryness and dissolve the residue in 0.5 mL of 1 mol/L hydrochloric acid TS and 5 mL of water. To 2 mL of this solution, add 5 mL of Reinecke salt TS: a pale red precipitate is produced.

(3) Determine the absorption spectra of solutions of Tipepidine Hibenzate and Tipepidine Hibenzate RS in ethanol (99.5) (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared spectra of Tipepidine Hibenzate and Tipepidine Hibenzate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 189 ~ 193 ºC.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Tipepidine Hibenzate in 10 mL of acetic acid (100): the solution is clear. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry: its absorbance at 400 nm is not more than 0.16.

(2) ***Heavy metals***—Proceed with 2.0 g of Tipepidine Hibenzate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Tipepidine Hibenzate according to Method 3 and perform the test (not more than 2 ppm).

(4) ***Related substances*** (i) Dissolve 10 mg of Tipepidine Hibenzate in 20 mL of the mobile phase and use this solution as the test solution. Pipet 1 mL of the test solution, add the mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of any peaks other than the peaks of hibenzic acid and tipepidine from the test solution is not larger than the peak area of the tipepidine from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Column temperature: A constant temperature of about 50 ºC.

Mobile phase: A mixture of a solution of ammonium acetate (1 in 100) and tetrahydrofuran (32 : 13).

Flow rate: Adjust the flow rate so that the retention time of tipepidine is between 10 and 14 minutes.

System suitability

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of tipepidine obtained from 20 μL of this solution is equivalent to 7 % to 13 % of that of tipepidine obtained from 20 μL of the standard solution.

System performance: Dissolve 10 mg of Tipepidine Hibenzate and 3 mg of propyl parahydroxybenzoate in 100 mL of the mobile phase. When the procedure is run with 20 μL of this solution under the above operating conditions, hibenzic acid, tipepidine and propyl parahydroxybenzoate are eluted in this order with a resolution between the peaks of tipepidine and propyl parahydroxybenzoate being not less than 3.0.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tipepidine is not more than 1.5 %.

Time span of measurement: A range of the retention time of tipepidine after the solvent peak.

(ii) Dissolve 10 mg of Tipepidine Hibenzate in 20 mL of the mobile phase and use this solution as the test solution. Pipet 1 mL of the test solution, add the mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of all peaks other than the area of the hibenzic acid and tipepidine from the test solution is not larger than half of the peak area of the tipepidine from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Column temperature: A constant temperature of about 40 ºC.

Mobile phase: A mixture of methanol and a solution of ammonium acetate (1 in 500) (13 : 7).

Flow rate: Adjust the flow rate so that the retention time of tipepidine is between 8 and 12 minutes.

System suitability

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of tipepidine obtained from 20 μL of this solution is equivalent to 7 % to 13 % of that of tipepidine obtained from 20 μL of the standard solution.

System performance: Dissolve 10 mg of Tipepidine Hibenzate and 3 mg of propyl parahydroxybenzoate in 100 mL of the mobile phase. When the procedure is run with 20 μL of this solution under the above operating conditions, hibenzic acid, tipepidine and propyl parahydroxybenzoate are eluted in this order with a resolution between the peaks of tipepidine and propyl parahydroxybenzoate being not less than 3.0.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tipepidine is not more than 1.5 %.

Time span of measurement: About twice as long as the retention time of tipepidine after the solvent peak.

Loss on Drying Not more than 0.5 % (1 g, 60 ºC, in vacuum, P_2O_5, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 1 g of Tipepidine Hibenzate, previously dried, dissolve in 40 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS. The end point of titration is only when the color of the solution changes from purple through blue to green (indicator: 3 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 51.766 mg of $C_{15}H_{17}NS_2C_{14}H_{10}O_4$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Tipepidine Hibenzate Tablets

Tipepidine Hibenzate Tablets contain not less than 95.0 % and not more than 105.0 % of the labeled amount of tipepidine hibenzate ($C_{15}H_{17}NS_2{\cdot}C_{14}H_{10}O_4$: 517.66).

Method of Preparation Prepare as directed under Tablets, with Tipepidine Hibenzate.

Identification (1) To a portion of powdered Tipepidine Hibenzate Tablets, equivalent to 44 mg of tipepidine hibenzate according to the labeled amount, add 5 mL of water, shake for 1 minute, add 10 mL of sodium hydroxide TS and extract with two 20 mL volumes of chloroform. Combine the extracts, wash with 10 mL of water and filter the chloroform layer. Evaporate the filtrate on a water-bath to dryness, dissolve the residue in 0.2 mL of 1 mol/L hydrochloric acid TS and 2 mL of water and add 5 mL of Reinecke salt TS: a pale red precipitate is produced.

(2) To a portion of powdered Tipepidine Hibenzate Tablets, equivalent to 11 mg of tipepidine hibenzate according to the labeled amount, add 30 mL of ethanol (99.5) and warm for 10 minutes with occasional shaking. After cooling, add ethanol (99.5) to make 50 mL and filter. To 1 mL of the filtrate, add ethanol (99.5) to make 20 mL and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 282 nm and 286 nm.

Dissolution Test Perform the test with 1 tablet of Tipepidine Hibenzate Tablets at 50 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of water as the dissolution solution. Use the dissolved solution after 30 minutes from starting of the test as the test solution. Separately, weigh accurately about 0.11 g of Tipepidine Hibenzate RS, previously dried in a desiccator (in vacuum, P_2O_5, 60 ºC, 3 hours) and dissolve in diluted ethanol (3 in 4) by warming occasionally. After cooling, add diluted ethanol (3 in 4) to make exactly 300 mL. Pipet 20 mL of this solution, add water to make exactly 900 mL and use this solu-

tion as the standard solution. Determine the absorbances, A_{T1} and A_{S1}, at 286 nm and A_{T2} and A_{S2}, at 360 nm of the test solution and the standard solution, respectively, as directed under Ultraviolet-visible Spectrophotometry.
The dissolution rate of Tipepidine Hibenzate Tablets in 30 minutes is not less than 80 %.

$$\text{Dissolution rate (\%) with respect to the labeled amount of tipepidine hibenzate } (C_{15}H_{17}NS_2{\cdot}C_{14}H_{10}O_4) = W_S \times \frac{A_{T1}\text{-}A_{T2}}{A_{S1}\text{-}A_{S2}} \times \frac{20}{C}$$

W_S: A mount (mg) of tipepidine hibenzate RS,
C: Labeled amount (mg) of tipepidine hibenzate ($C_{15}H_{17}NS_2{\cdot}C_{14}H_{10}O_4$) in 1 tablet.

Uniformity of Dosage Units Perform the test according to the following method: it meets the requirement of the Content uniformity test.
To 1 tablet of Tipepidine Hibenzate Tablets add 5 mL of diluted acetic acid (100) and 15 mL of methanol for each 11 mg of tipepidine hibenzate, and warm for 15 minutes with occasional shaking. After cooling, add diluted methanol solution (1 in 2) to make exactly V mL so that each mL contains about 0.44 mg of tipepidine hibenzate, filter, discard the first 10 mL of the filtrate, and pipet 5 mL of the subsequent filtrate. Add 5 mL of the internal standard solution, add diluted methanol solution (1 in 2) to make exactly 25 mL, and use this solution as the test solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of tipepidine hibenzate } (C_{15}H_{17}NS_2{\cdot}C_{14}H_{10}O_4) = \text{Amount (mg) of Tipepidine Hibenzate RS} \times \frac{Q_T}{Q_S} \times \frac{V}{50}$$

Internal standard solution—A solution of dibucaine hydrochloride in methanol (1 in 2000)

Assay Weigh accurately and powder not less than 20 Tipepidine Hibenzate Tablets. Weigh accurately a portion of the powder, equivalent to about 22 mg of tipepidine hibenzate ($C_{15}H_{17}NS_2{\cdot}C_{14}H_{10}O_4$), add 10 mL of diluted acetic acid (100) (1 in 2) and 30 mL of methanol and warm for 10 minutes with occasional shaking. After cooling, add diluted methanol (1 in 2) to make exactly 50 mL and filter. Discard the first 10 mL of the filtrate, pipet 5.0 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution, then add diluted methanol (1 in 2) to make exactly 25 mL and use this solution as the test solution. Separately, weigh accurately about 22 mg of Tipepidine Hibenzate RS, previously dried in a desiccator (in vacuum, P_2O_5, 60 ºC, 3 hours), dissolve in 10 mL of diluted acetic acid (100) (1 in 2) and 30 mL of methanol and add diluted methanol (1 in 2) to make exactly 50 mL. Pipet 5.0 mL of this solution, add 5.0 mL of the internal standard solution, then add diluted methanol (1 in 2) to make exactly 25 mL and use this solution as the standard solution. Perform the test with 20 µL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of tipepidine to that of the internal standard, for the test solution and the standard solution, respectively.

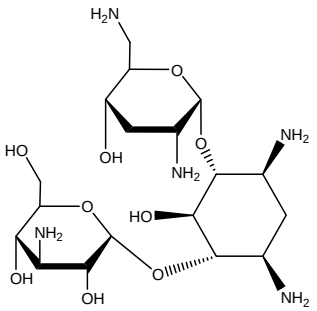
$$\text{Amount (mg) of tipepidine hibenzate } (C_{15}H_{17}NS_2{\cdot}C_{14}H_{10}O_4) = \text{Amount (mg) of Tipepidine Hibenzate RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of dibucaine hydrochloridc in methanol (1 in 2000).

Operating conditions
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column, about 4 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm to 10 µm in particle diameter).
Column temperature: A constant temperature of about 40 ºC.
Mobile phase: A mixture of a solution of sodium lauryl sulfate in diluted phosphoric acid (1 in 1000) (1 in 500), acetonitrile and 2-propanol (3 : 2 : 1).
Flow rate: Adjust the flow rate so that the retention time of Tipepidine is about 7 minutes.
System suitablilty
System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, tipepidine and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.0.
System repeatability: When the test is repeated 6 times with 20 µL each of standard solution under the above operating conditions: the relative standard deviation of the peak area of tipepidine is not more than 3.0 %.

Containers and Storage ***Containers***—Tight containers.
Storage—Light-resistant.

Tobramycin



$C_{18}H_{37}N_5O_9$: 467.51

(2*S*,3*R*,4*S*,5*S*,6*R*)-4-Amino-2-[(1*S*,2*S*,3*R*,4*S*,6*R*)-4,6-diamino-3-[(2*R*,3*R*,5*S*,6*R*)-3-amino-6-(aminomethyl)-5-hydroxyoxan-2-yl]oxy-2-hydroxycyclohexyl]oxy-6-(hydroxymethyl)oxane-3,5-diol [*32986-56-4*]

Tobramycin is an aminoglycoside substance having antibacterial activity produced by the growth of *Streptomyces tenebrarius*.
Tobramycin contains not less than 900 μg (potency) and not more than 1060 μg (potency) per mg of tobramycin ($C_{18}H_{37}N_5O_9$), calculated on the anhydrous basis

Description Tobramycin appears as white to pale yellowish white powder.
Tobramycin is very soluble in water, freely soluble in formamide, slightly soluble in methanol, and very slightly soluble in ethanol (95).
Tobramycin is hygroscopic.

Identification (1) Dissolve 10 mg each of Tobramycin and Tobramycin RS in 1 mL of water, and use these solutions as the test solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 4 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ammonia TS, 1-butanol, and methanol (5 : 5 : 2) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin TS on the plate, and heat at 100 °C for 5 mintues: the principal spot obtained from the test solution shows the same R_f value as the spot from the standard solution.

(2) Determine the ^1H spectrum of a solution of Tobramycin in heavy water for nuclear magnetic resonance spectroscopy (1 in 125) as directed under Nuclear Magnetic Resonance Spectroscopy, using sodium 3-trimethyl-silylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a double signal, A, at around δ 5.1 ppm, a multiple signal, B, at around δ 2.6 to 4.0 ppm, and a multiple signal, C, at around δ 1.0 to 2.1 ppm. The ratio of the integrated intensity of these signals, A : B : C, is 1 : 8 : 2.

Specific Optical Rotation $[\alpha]_D^{20}$: +138 ~ +148° (1 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.10 g of Tobramycin in 10 mL of water is between 9.5 and 11.5.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Tobramycin in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) ***Heavy metals***—Proceed with 1.0 g of Tobramycin according to Method 2 and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

(3) ***Related substances***—Dissolve 80 mg of Tobramycin in 10 mL of diluted ammonia solution (28) (1 in 250), and use this solution as the test solution. Pipet 1 mL of the test solution, add diluted ammonia solution (28) (1 in 250) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ammonia solution (28), ethanol (95), and 2-butanone (1 : 1 : 1) to a distance of about 10 cm, air-dry the plate, and dry again at 110 °C for 10 minutes. Immediately spray a mixture of water and sodium hypochlorite TS (4 : 1) on the plate, air-dry the plate, and spray potassium iodide-starch TS: any spot other than the principal spot obtained from the test solution is not more intense than the spot from the standard solution.

Water Not more than 11.0 % (0.1 g, volumetric titration, direct titration). Use a mixture of formamide for water determination and methanol for water determination (3 : 1) instead of methanol for water determination.

Residue on Ignition Not more than 1.0 % (0.5 g).

Bacterial Endotoxins Less than 2.0 EU/mg (potency) of tobramycin, when Tobramycin is used in a sterile preparation.

Assay Weigh accurately about 50 mg (potency) each of Tobramycin and Tobramycin RS, dissolve each in water to make exactly 250 mL, and ues these solutions as the test solution and standard solution, respectively. Pipet 4.0 mL each of the test solution and standard solution, transfer to separate 50 mL volumetric flasks, add 10 mL of 2,4-dinitrofluorobenzene TS and 10 mL of 2-amino-2-hydroxymethyl-1,3-propandiol TS, shake, allow to stand in a water bath at 60 ± 2 °C for 50 ± 5 minutes, and allow to stand at room temperature for 10 minutes. Add about 20 mL of acetonitrile, cool to room temperature, add acetonitrile to make exactly 50 mL, filter through a membrane filter with a pore size not exceeding 0.5 μm, and use the filtrates as the derivatized test solution and derivatized standard solution, respectively. Perform the test with 20 μL each of the derivatized test solution and derivatized standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of tobramycin in each solution.

$$\text{Amount [μg (potency)] of tobramycin } (C_{18}H_{37}N_5O_9) = \text{Amount [μg (potency)] of Tobramycin RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 365 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Mobile phase: Dissolve 2.0 g of 2-amino-2-hydroxymethyl-1,3-propandiol in 800 mL of water. To this solution add 20 mL of 0.5 mol/L sulfuric acid solution and acetonitrile to make exactly 2000 mL.

Flow rate: 1.2 mL/minute

System suitability

System performance: Weigh accurately 5 mg of *p*-naphtholbenzeine, add acetonitrile to make exactly 20 mL, to 2 mL of this solution add the derivatized standard solution to make 10 mL, and use this solution as the system suitability solution. When the system suitability solution is injected, the relative retention time of *p*-naphtholbenzine with respect to tobramycin is about 0.6 with the resolution between these peaks being not less than 4.0.

Containers and Storage ***Containers***—Tight containers.

Tobramycin Injection

Tobramycin Injection contains not less than 90.0 % and not more than 110.0 % of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$: 467.52).

Method of Preparation Prepare as directed under Injections, with Tobramycin.

Description Tobramycin Injection appears as a clear, colorless to pale yellow liquid.

Identification Pipet a volume of Tobramycin Injection, equivalent to about 10 mg (potency), add water to make exactly 1 mL, and use this solution as the test solution. Separately, weigh accurately 10 mg (potency) of Tobramycin RS, add water to make exactly 1 mL, and use this solution as the standard solution. Perform the test with the test solution and standard solution as directed under Thin-layer Chromatography. Spot 4 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ammonia water, 1-butanol, and methanol (5 : 5 : 2) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin TS on the plate, and heat at 100 °C for 5 minute: the spots obtained from the test solution and standard solution have the same R_f value.

pH 5.0 ~ 7.0.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.50 EU/mg (potency) of tobramycin.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay Proceed as directed in the Assay under Tobramycin. Take a volume of Tobramycin Injection, equivalent to about 50 mg (potency) according to the labeled petency, add water to make exactly 250 mL, and use this solution as the test solution.

Containers and Storage ***Containers***—Hermetic containers.

Tobramycin Ophthalmic Ointment

Tobramycin Ophthalmic Ointment contains not less than 90.0 % and not more than 120.0 % of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$: 467.52).

Method of Preparation Prepare as directed under Ophthalmic Ointments, with Tobramycin.

Identification Proceed as directed in the Identification (1) under Tobramycin. Transfer a suitable amount of Tobramycin Ophthalmic Ointment to a separatory funnel, dissolve in 10 mL of chloroform, add water, shake, allow to stand until the two liquid layers are separated completely, take the water layer, dilute with water to that each mL contains 3 mg (potency), and use this solution as the test solution. Separately, dissolve a suitable amount of Tobramycin RS so that each mL contains 3 mg (potency), and use this solution as the standard solution. Spot 5 μL of the test solution and standard solution.

Water Not more than 1.0 % (1.0 g, volumetric titration, direct titration).

Sterility Test It meets the requirement.

Test for Metal Particles It meets the requirement.

Assay Proceed as directed in the Assay under Tobramycin. Weigh accurately a suitable amount of Tobramycin Ophthalmic Ointment, transfer to a separatory funnel, add 50 mL of ether, extract with three 25 mL volumes of sterile purified water, combine the extracts, and add sterile purified water to make exactly 100 mL. Pipet a suitable volume of this solution, dilute with sterile purified water so that each mL contains 0.2 mg (potency), and use this solution as the test solution.

Containers and Storage ***Containers***—Tight containers.

Tobramycin Ophthalmic Solution

Tobramycin Ophthalmic Solution contains not less than 90.0 % and not more than 120.0 % of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$: 467.52).

Method of Preparation Prepare as directed under Ophthalmic Solutions, with Tobramycin.

Identification Proceed as directed in Identification (1) under Tobramycin. Weigh accurately a suitable amount each of Tobramycin Ophthalmic Solution and Tobramycin RS, add water so that each mL contains 3 mg (potency), and use these solutions as the test solution and standard solution, respectively.

pH 7.0 ~ 8.0.

Sterility Test It emets the requirement.

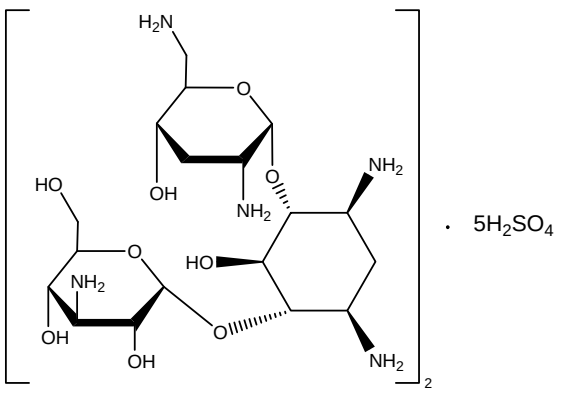
Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Ophthalmic Solutions It meets the requirement.

Assay Proceed as directed in the Assay under Tobramycin. Dissolve an amount of Tobramycin Ophthalmic Solution, equivalent to about 50 mg (potency) according to the labeled potency, in water to make exactly 250 mL, and use this solution as the test solution.

Containers and Storage ***Containers***—Tight containers.

Tobramycin Sulfate



$(C_{18}H_{37}N_5O_9)_2 \cdot 5H_2SO_4$: 1425.43

(2*S*,3*R*,4*S*,5*S*,6*R*)-4-Amino-2-[(1*S*,2*S*,3*R*,4*S*,6*R*)-4,6-diamino-3-[(2*R*,3*R*,5*S*,6*R*)-3-amino-6-(aminomethyl)-5-hydroxyoxan-2-yl]oxy-2-hydroxycyclohexyl]oxy-6-(hydroxymethyl)oxane-3,5-diol;sulfuric acid [*49842-07-1*]

Tobramycin Sulfate contains not less than 634 μg (potency) and not more than 739 μg (potency) per mg of tobramycin ($C_{18}H_{37}N_5O_9$: 467.52), calculated on the anhydrous basis.

Description Tobramycin Sulfate appears as white to pale yellowish white powder.
Tobramycin Sulfate is freely soluble in water, slightly soluble in ethanol (95), and practically insoluble in chloroform or in ether.
Tobramycin Sulfate is hygroscopic.

Identification (1) Proceed as directed in Identification under Tobramycin. Dissolve about 60 mg (potency) each of Tobramycin Sulfate and Tobramycin RS in water so that each mL contains 6 mg (potency), and use these solutions as the test solution and standard solution, respectively.

(2) The retention time of the principal peak obtained from the test solution corresponds to that from the standard solution, as obtained in the Assay.

(3) Tobramycin Sulfate responds to the Qualitative Tests for sulfate.

pH Dissolve 0.4 g (potency) of Tobramycin Sulfate in 10 mL of water: the pH of the solution is between 6.0 and 8.0.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Tobramycin Sulfate according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

(2) ***Related substances***—Weigh accurately 50 mg of Tobramycin Sulfate, dissolve in 7 mL of water, adjust the pH to 5.5 with 1 mol/L sulfuric acid, add water to make 10 mL, and use this solution as the test solution. Pipet 1 mL of the test solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the test solution and standard solution as directed under Thin-layer Chromatography. Spot 1 μL each of the test solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of sodium chloride solution (29.2 in 100), alcohol, and water (50 : 30 : 20) to a distance of about 15 cm, dry the plate in warm air, and heat at 110 °C for 10 minutes. Spray diluted sodium hypochlorite TS (1 in 5) on the hot plate, and stand in cool air until the addition of one drop of potassium iodide-starch TS below the origin line on the plate produces a very pale blue color. Spray evenly potassium iodide-starch TS on the plate: a blue-purple spot appears. Any spot other than the principal spot obtained from the test solution is not more intense than the spot from the standard solution (not more than

1.0 %).

Water Not more than 2.0 % (0.5 g, volumetric titration, direct titration).

Residue on Ignition Not more than 1.0 % (1 g).

Sterility Test It meets the requirement, when Tobramycin Sulfate is used in a sterile preparation.

Bacterial Endotoxins Less than 2.0 EU/mg (potency) of tobramycin, when Tobramycin Sulfate is used in a sterile preparation.

Assay Weigh accurately about 50 mg (potency) each of Tobramycin Sulfate and Tobramycin RS, dissolve each in water to make exactly 250 mL, and use these solutions as the test solution and standard solution, respectively. Pipet 4.0 mL each of the test solution and standard solution, transfer to separate 50 mL volumetric flasks, add 10 mL of 2,4-dinitrobenzene TS and 10 mL of 2-amino-2-hydroxymethyl-1,3-propandiol TS, shake, allow to stand in a water bath at 60 ± 2 °C for 50 ± 5 minutes, and allow to stand for 10 minutes at room temperature. Add about 20 mL of acetonitrile, cool to room temperature, add acetonitrile to make exactly 50 mL, filter through a membrane filter with a pore size not exceeding 0.5 μm, and use the filtrates as the derivatized test solution and derivatized standard solution, respectively. Perform the test with 20 μL each of the derivatized test solution and derivatized standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of tobramycin in each solution.

$$\text{Amount [μg (potency)] of tobramycin } (C_{18}H_{37}N_5O_9) = \text{Amount [μg (potency)] of Tobramycin RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 365 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Mobile phase: Dissolve 2.0 g of 2-amino-2-hydroxymethyl-1,3-propandiol in 800 mL of water. To this solution add 20 mL of 1 mol/L sulfuric acid solution and acetonitrile to make exactly 2000 mL.

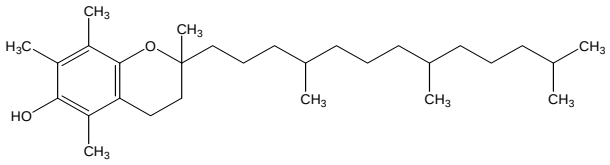
Flow rate: 1.2 mL/minute

System suitability

System performance: Weigh accurately 5 mg of *p*-naphtholbenzeine, add acetonitrile to make exactly 20 mL, to 2 mL of this solution add the derivatized standard solution to make 10 mL, and use this solution as the system suitability solution. When the procedure is run with the system suitability solution, the relative retention time of *p*-naphtholbenzine with respect to tobramycin is about 0.6 with the resolution between these peaks being not less than 4.0.

Containers and Storage ***Containers***—Tight containers.

Tocopherol



Vitamin E

dl-α-Tocopherol $C_{29}H_{50}O_2$: 430.71

(2*R*)-2,5,7,8-Tetramethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-3,4-dihydrochromen-6-ol

[*10191-41-0*]

Tocopherol contains not less than 96.0 % and not more than 102.0 % of *dl*-α-tocopherol ($C_{29}H_{50}O_2$).

Description Tocopherol is a clear, yellow to red-brown, viscous liquid and odorless.

Tocopherol is miscible with ethanol (99.5), with ether, with chloroform or with vegetable oils.

Tocopherol is freely soluble in ethanol (95) and practically insoluble in water.

Tocopherol shows no optical rotation.

Tocopherol is oxidized by air and light and acquires a dark red color.

Identification (1) Dissolve 10 mg of Tocopherol in 10 mL of ethanol (99.5), add 2 mL of nitric acid and heat at 75 °C for 15 minutes: a red to orange color is observed.

(2) Determine the infrared spectra of Tocopherol and Tocopherol RS as directed in the liquid film method under Infrared Spectrophotometry: both spectra exhibit the similar intensities of absorption at the same wavenumbers.

Refractive index n_D^{20} : 1.503 ~ 1.507.

Specific gravity d_{20}^{20} : 0.947 ~ 0.955.

Absorbance $E_{1cm}^{1\%}$ (292 nm): 71.0 ~ 76.0 (10 mg, ethanol (99.5), 200 mL).

Purity (1) ***Clarity and color of solution***—Dissolve 0.10 g of Tocopherol in 10 mL of ethanol (99.5): the solution is clear and has no more color than Color Matching Fluid C.

(2) ***Heavy metals***—Proceed with 1.0 g of Tocopherol according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

Assay Dissolve about 50 mg each of Tocopherol and Tocopherol RS, accurately weighed, in ethanol (99.5) to make exactly 50 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and determine the peak heights, H_T and H_S, of Tocopherol in the test solution and the standard solution, respectively.

$$\text{Amount (mg) of tocopherol } (C_{29}H_{50}O_2) = \text{Amount (mg) of Tocopherol RS} \times \frac{H_T}{H_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 292 nm).

Column: A stainless steel column, about 4 mm in internal diameter and 15 cm to 30 cm in length, packed with octadecylsilanized silica gel (5 to μm 10 μm in particle diameter).

Mobile phase: A mixture of methanol and water (49 : 1).

Flow rate: Adjust the flow rate so that the retention time of Tocopherol is about 10 minutes.

System suitability

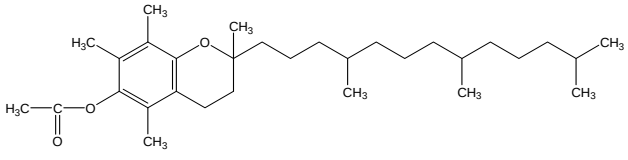
System performance: Dissolve 50 mg each of Tocopherol and Tocopherol Acetate in 50 mL of ethanol (99.5). When the procedure is run with 20 μL of this solution under the above operating conditions, tocopherol and tocopherol acetate are eluted in this order with the resolution between these peaks being not less than 2.6.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions: the relative standard deviation of the peak area of tocopherol is not more than 0.8 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and well-filled or under nitrogen atmosphere.

Tocopherol Acetate



Vitamin E Acetate

dl-α-Tocopherol Acetate $C_{31}H_{52}O_3$: 472.74

[(2*R*)-2,5,7,8-Tetramethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-3,4-dihydrochromen-6-yl]acetate [*7695-91-2*]

Tocopherol Acetate contains not less than 96.0 % and not more than 102.0 % of *dl*-α-tocopherol acetate ($C_{31}H_{52}O_3$).

Description Tocopherol Acetate is a clear, colorless to yellow, viscous liquid and is odorless.

Tocopherol Acetate is miscible with ethanol (99.5), with acetone, with chloroform, with ether, with hexane or with fried oils.

Tocopherol Acetate is freely soluble in ethanol (95) and practically insoluble in water.

Tocopherol Acetate shows no optical rotation.

Tocopherol Acetate is affected by air and light.

Identification (1) Dissolve 50 mg of Tocopherol Acetate in 10 mL of ethanol (99.5), add 2 mL of nitric acid and heat at 75 °C for 15 minutes: a red to orange color is observed.

(2) Determine the infrared spectra of Tocopherol Acetate and Tocopherol Acetate RS as directed in the liquid film method under Infrared Spectrophotometry: both spectra exhibit the similar intensity of absorption at the same wavenumbers.

Refractive Index n_D^{20} : 1.494 ~ 1.499.

Specific gravity d_{20}^{20} : 0.952 ~ 0.966.

Absorbance $E_{1cm}^{1\%}$ (284 nm): 41.0 ~ 45.0 (10 mg, ethanol (99.5), 100 mL).

Purity (1) ***Clarity and color of solution***—Dissolve 0.10 g of Tocopherol Acetate in 10 mL of ethanol (99.5): the solution is clear and has no more color than the following control solution.

Control solution—To 0.5 mL of iron (III) chloride colorimetric stock solution, add 0.5 mol/L hydrochloric acid TS to make 100 mL.

(2) ***Heavy metals***—Carbonize 1.0 g of Tocopherol Acetate by gentle heating. After cooling, add 10 mL of a solution of magnesium nitrate in ethanol (95) (1 in 10) and ignite the ethanol to burn. After cooling, add 1 mL of sulfuric acid, proceed according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***α-Tocopherol***—Dissolve 0.10 g of Tocopherol Acetate in exactly 10 mL of hexane and use this solu-

tion as the test solution. Separately, dissolve 50 mg of Tocopherol RS in hexane to make exactly 100 mL. Pipet 1.0 mL of this solution, add hexane to make exactly 10 mL and use this solution as the standard solution. Perform the test with the test solution at the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of toluene and acetic acid (100) (19 : 1) to a distance of about 10 cm and air-dry the plate. Spray evenly a solution of iron (III) chloride in ethanol (99.5) (1 in 500) on the plate, then spray evenly a solution of 2,2'-dipyridyl in ethanol (99.5) (1 in 200) on the same plate and allow to stand for 2 to 3 minutes: the spot from the test solution corresponding to that from the standard solution is not larger than and not more intense than the spot from the standard solution.

Assay Dissolve 50 mg each of Tocopherol Acetate and Tocopherol Acetate RS, accurately weighed, in ethanol (99.5) to make exactly 50 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions and determine the peak heights, H_T and H_S, of Tocopherol Acetate in the test solution and the standard solution, respectively.

$$\text{Amount (mg) of tocopherol acetate } (C_{31}H_{52}O_3) = \text{Amount (mg) of Tocopherol Acetate RS} \times \frac{H_T}{H_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 284 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel (5 μm in particle diameter).

Column temperature: A constant temperature of about 35 °C.

Mobile phase: A mixture of methanol and water (49 : 1).

Flow rate: Adjust the flow rate so that the retention time of Tocopherol Acetate is about 12 minutes.

System suitability

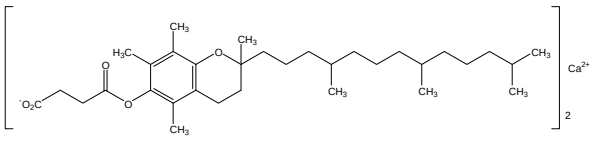
System performance: Dissolve 50 mg each of Tocopherol Acetate and Tocopherol in 50 mL of ethanol (99.5). When the procedure is run with 20 μL of this solution under the above operating conditions, tocopherol and tocopherol acetate are eluted in this order with the resolution between these peaks being not less than 2.6.

System repeatability: When the test is repeated 5 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tocopherol acetate is not more than 0.8 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Tocopherol Calcium Succinate



Vitamin E Calcium Succinate $C_{66}H_{106}CaO_{10}$: 1099.62

Calcium 4-oxo-4-[[(2*R*)-2,5,7,8-tetramethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-3,4-dihydrochromen-6-yl]oxy]butanoate [*14638-18-7*]

Tocopherol Calcium Succinate, when dried, contains not less than 96.0 % and not more than 102.0 % of *dl*-α-tocopherol calcium succinate ($C_{66}H_{106}CaO_{10}$).

Description Tocopherol Calcium Succinate is a white to yellowish white powder and is odorless.

Tocopherol Calcium Succinate is freely soluble in chloroform or in carbon tetrachloride and practically insoluble in water, in ethanol (95) or in acetone.

Shake 1 g of Tocopherol Calcium Succinate with 7 mL of acetic acid (100): it dissolves and produces a turbidity after being allowed to stand for a while.

Tocopherol Calcium Succinate dissolves in acetic acid (100).

Tocopherol Calcium Succinate shows no optical rotation.

Identification (1) Dissolve 50 mg of Tocopherol Calcium Succinate in 1 mL of acetic acid (100), add 9 mL of ethanol (99.5) and mix. To this solution, add 2 mL of fuming nitric acid and heat at 75 ºC for 15 minutes: a red to orange color is observed.

(2) Dissolve 80 mg each of Tocopherol Calcium Succinate and Tocopherol Calcium Succinate RS, previously dried, in 0.2 mL of carbon tetrachloride and determine the infrared spectra of these solutions as directed in the liquid film method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Dissolve 5 g of Tocopherol Calcium Succinate in 30 mL of chloroform, add 10 mL of hydrochloric acid, shake for 10 minutes, then draw off the water layer and neutralize with ammonia TS: the solution responds to the Qualitative Tests for calcium salt.

Absorbance $E_{1\text{cm}}^{1\%}$ (286 nm): 36.0 ~ 40.0 (10 mg, chloroform, 100 mL).

Purity (1) ***Clarity and color of solution***—Dissolve 0.10 g of Tocopherol Calcium Succinate in 10 mL of

chloroform: the solution is clear and has no more color than the following control solution.

Control solution—To 0.5 mL of iron (III) chloride colorimetric stock solution, add 0.5 mol/L hydrochloric acid TS to make 100 mL.

(2) ***Alkali***—To 0.20 g of Tocopherol Caicium Succinate, add 10 mL of ether, 2 mL of water, 1 drop of phenolphthalein TS and 0.10 mL of 0.1 mol/L hydrochloric acid VS and shake: no red color is observed in the water layer.

(3) ***Chloride***—Dissolve 0.10 g of Tocopherol Calcium Succinate in 4 mL of acetic acid (100), add 20 mL of water and 50 mL of ether, shake thoroughly and collect the water layer. To the ether layer, add 10 mL of water, shake and collect the water layer. Combine the water layers, add 6 mL of dilute nitric acid and water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution in the same manner using 0.60 mL of 0.01 mol/L hydrochloric acid VS in place of Tocopherol Calcium Succinate (not more than 0.212 %).

(4) ***Heavy metals***—Proceed with 1.0 g of Tocopherol Calcium Succinate according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(5) ***Arsenic***—Prepare the test solution with 1.0 g of Tocopherol Calcium Succinate according to Method 3 and perform the test (not more than 2 ppm).

(6) ***α-Tocopherol***—Dissolve 0.10 g of Tocopherol Calcium Succinate in chloroform to make exactly 10 mL and use this solution as the test solution. Separately, dissolve 50 mg of Tocopherol RS in chloroform to make exactly 100 mL. Pipet 1.0 mL of this solution, add chloroform to make exactly 10 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of toluene and acetic acid (100) (19 : 1) to a distance of about 10 cm and air-dry the plate. Spray evenly a solution of iron (III) chloride hexahydrate in ethanol (99.5) (1 in 500) on the plate, then spray evenly a solution of 2,2'-dipyridyl in ethanol (99.5) (1 in 200) on the same plate and allow to stand for 2 to 3 minutes: the spots from the test solution corresponding to the spots from the standard solution is not larger than and not more intense than the spots from the standard solution.

Loss on Drying Not more than 2.0 % (1 g, in vacuum, P_2O_5, 24 hours).

Assay Weigh accurately about 50 mg each of Tocopherol Calcium Succinate and tocopherol succinate RS, previously dried, dissolve in a mixture of ethanol (99.5) and diluted acetic acid (100) (1 in 5) (9 : 1) to make exactly 50 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. Determine the peak heights, H_T and H_S, of tocopherol succinate for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of tocopherol calcium succinate } (C_{66}H_{106}CaO_{10}) = \text{Amount (mg) of Tocopherol Succinate RS} \times \frac{H_T}{H_S} \times 1.0358$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 284 nm).

Column: A stainless steel column, about 4 mm in internal diameter and 15 cm to 30 cm in length, packed with octadecylsilanized silica gel (5 μm to 10 μm in particle diameter).

Mobile phase: A mixture of methanol, water and acetic acid (100) (97 : 2 : 1).

Flow rate: Adjust the flow rate so that the retention time of tocopherol succinate is about 8 minutes.

System suitability

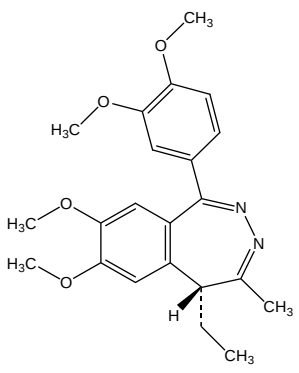
System performance: Dissolve 50 mg each of tocopherol succinate and Tocopherol in 50 mL of a mixture (9 : 1) of ethanol (99.5) and diluted acetic acid (100) (1 in 5). When the procedure is run with 20 μL of this solution under the above operating conditions, tocopherol succinate and tocopherol are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL each of standard solution under the above operating conditions, the relative standard deviation of the peak area of tocopherol succinate is not more than 0.8 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Tofisopam



and enantiomer

$C_{22}H_{26}N_2O_4$: 382.45

1-(3,4-Dimethoxyphenyl)-5-ethyl-7,8-dimethoxy-4-methyl-5*H*-2,3-benzodiazepine [*22345-47-7*]

Tofisopam, when dried, contains not less than 98.0 % and not more than 101.0 % of tofisopam ($C_{22}H_{26}N_2O_4$).

Description Tofisopam is a pale yellowish white, crystalline powder.
Tofisopam is freely soluble in acetic acid (100), soluble in acetone, sparingly soluble in ethanol (95), slightly soluble in ether, and practically insoluble in water.
A solution of Tofisopam in ethanol (95) (1 in 100) shows no optical rotation.

Identification (1) Determine the absorption spectra of solutions of Tofisopam and Tofisopam RS in ethanol (95) (1 in 100000) as directed under Ultraviolet-visible Spcctrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Tofisopam and Tofisopam RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 155 ~ 159 °C.

Purity (1) ***Heavy metals***—Procced with 1.0 g of Tofisopam according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Arsenic***—Prepare the test solution with 1.0 g of Tofisopam according to Method 3, and perform the test (not more than 2 ppm).

(3) ***Related substances***—Dissolve 50 mg of Tofisopam in 10 mL of acetone, and use this solution as the test solution. Pipet 1 mL of the test solution, add acetone to make exactly 25 mL. Pipet 1 mL of this solution, add acetone to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, methanol and formic acid (24 : 12 : 2 : 1) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, silica gel, 60 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

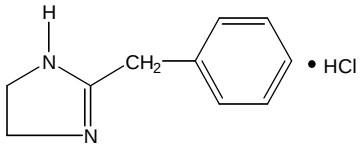
Assay Weigh accurately about 0.2 g of Tofisopam, previously dried, dissolve in 50 mL of acetic acid (100), and titrate with 0.1 mnol/L perchloric acid VS (poterntiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 38.25 mg of $C_{22}H_{26}N_2O_4$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Tolazoline Hydrochloride



$C_{10}H_{12}N_2 \cdot HCl$: 196.68

2-Benzyl-4,5-dihydro-1*H*-imidazolehydrochloride [*59-97-2*]

Tolazoline Hydrochloride contains not less than 98.0 % and not more than 101.0 % of tolazoline hydrochloride ($C_{10}H_{12}N_2 \cdot HCl$), calculated on the dried basis.

Description Tolazoline Hydrochloride is a white to grayish white crystalline powder.
Tolazoline Hydrochloride is freely soluble in water or in ethanol (95).
An aqueous solution of Tolazoline Hydrochloride is a weak acid, as tested with litmus paper.

Identification (1) Determine the infrared spectra of Tolazoline Hydrochloride and Tolazoline Hydrochloride RS as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit the similar intensities of absorption at the same wavenumbers.

(2) Perform the test with the identification solution used in the Related substances: The R_f value of the principal spot from the identification solution corresponds to the R_f value of the spot from the standard solution A.

Melting Point 172.0 ~ 176.0 °C.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Tolazoline Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Weigh accurately a portion of Tolazoline Hydrochloride, dissolve in methanol to obtain a solution containing 20 mg per mL and use this solution as the test solution. Dilute a volume of the

test solution quantitatively with methanol to obtain a solution containing 100 μg per mL and use this solution as the identification solution. Separately, weigh accurately a portion of Tolazoline Hydrochloride RS, previously dried (silica gel, in vacuum, 4 hours), dissolve in methanol to obtain a solution containing 100 μg per mL and use this solution as the standard solution (1). Pipet 4.0 mL, 3.0 mL, 2.0 mL and 1.0 mL of this solution, add methanol to make 5 mL and use these solutions as the standard solution (2), the standard solution (3), the standard solution (4) and the standard solution (5), respectively. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot separately 5 μL each of the test solution, the identification solution and the standard solutions (1) to (5) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia water (95 : 5) to a distance of about 15 cm and air-dry the plate for at least 30 min. Expose the plate to chlorine gas for not more than 5 minutes and air-dry until the chlorine has dissipated. Spray the detection reagent on the plate and compare the intensities of any secondary spots observed in the chromatogram of the test solution with those of the principal spots in the chromatograms of the standard solution: the sum of the intensities of all secondary spots obtained from the test solution is not more than 1.0 %, as compared with the intensity of the principal spot from the standard solution.

Detection reagent—Dissolve 0.5 g of potassium iodide in 50 mL of water (solution A). Dissolve 1.5 g of soluble starch in 50 mL of boiling water (solution B). Mix 10 mL each of the solutions A and B, and add 3 mL of ethanol (95) before use.

Loss on Drying Not more than 0.2 % (1 g, in vacuum, silica gel, 4 hours).

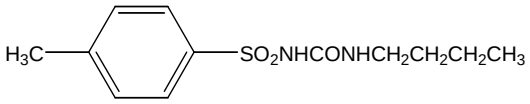
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.3 g of Tolazoline Hydrochloride, dissolve in 100 mL of acetic acid (100), add 25 mL of mercury (II) acetate TS and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid
= 19.668 mg of $C_{10}H_{12}N_2 \cdot HCl$.

Containers and Storage ***Containers***—Well-closed containers.

Tolbutamide



$C_{12}H_{18}N_2O_3S$: 270.35

3-Butyl-1-[(4-methylbenzene)sulfonyl]urea [*64-77-7*]

Tolbutamide, when dried, contains not less than 99.0 % and not more than 101.0 % of tolbutamide ($C_{12}H_{18}N_2O_3S$).

Description Tolbutamide appears as white crystals or crystalline powder, is odorless or has a slight, characteristic odor and is tasteless.
Tolutamide is soluble in ethanol (95), slightly soluble in ether and practically insoluble in water.

Identification (1) Boil 0.2 g of Tolbutamide with 8 mL of diluted sulfuric acid (1 in 3) under a redux condenser for 30 minutes. Cool the solution in ice-water, collect the precipitated crystals, recrystallize from water and dry at 105 °C for 3 hours: the crystals melt between 135 °C and 139 °C.

(2) To the filtrate obtained in (1) add about 20 mL of a solution of sodium hydroxide (1 in 5) to make alkaline and heat: an ammonia-like odor is perceptible.

Melting Point 126 ~ 132 °C.

Purity (1) ***Clarity of solution***—Dissolve 0.5 g of Tolbutamide in 10 mL of 0.5 mol/L ammonia water: the solution is clearer than milky white.

(2) ***Acid***—To 3.0 g of Tolbutamide, add 150 mL of water, warm at 70 °C for 5 minutes, allow to stand for 1 hour in ice-water and filter. To 25 mL of the filtrate, add 2 drops of methyl red TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: a yellow color is observed.

(3) ***Chloride***—To 40 mL of the filtrate obtained in (1), add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011 %).

(4) ***Sulfate***—To 40 mL of the filtrate obtained in (1), add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.021 %).

(5) ***Heavy metals***—Proceed with 2.0 g of Tolbutamide according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(6) ***Selenium***—To 0.1 g of Tolbutamide add 0.1 g of magnesium oxide, mix, transfer to a combustion flask, and proceed as directed under Oxygen Flask Combustion Method, using 25 mL of diluted nitric acid (1 in 30) as the absorbing liquid. Use a 1 L combustion

flask. After combustion, wash the stopper and the inside of the flask with 10 mL of water. Transfer the liquid inside the flask to a 150 mL beaker, using about 20 mL of water. Heat gently to boil, boil for 10 minutes, cool to room temperature, and use this solution as the test solution. Separately, pipet 3.0 mL of selenium standard stock solution, add 25 mL of diluted nitric acid (1 in 30) and 25 mL of water, and use this solution as the standard solution. Adjust the pH of the test solution and standard solution to 2.0 ± 0.2 with diluted ammonia solution (28) (1 in 2), add water to make exactly 60 mL, transfer to a separatory funnel using 10 mL of water, and wash the separatory funnel with 10 mL of water. Add 0.2 g of hydroxylamine hydrochloride, stir to dissolve, add immediately 5.0 mL of 2,3-diamino-naphthalene TS, stopper, stir to mix, and allow to stand at room temperature for 100 minutes. Add 5.0 mL of cyclohexane, shake vigorously for 2 minutes, allow the layers to separate, discard the water layer, centrifuge the cyclohexane extract to remove water, and take the cyclohexane layer. Determine the absorbances at about 380 nm of these solutions as directed under Ultraviolet-visible Spectrophotometry, using a solution prepared by adding 25 mL of water to 25 mL of diluted nitric acid (1 in 30) and proceeding in the same manner, as the blank: the absorbance obtained from the test solution is not more than that from the standard solution (not more than 30 ppm).

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

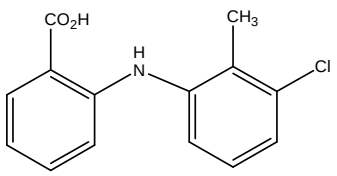
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.5 g of Tolbutamide, previously dried and dissolve in 30 mL of neutralized ethanol. Add 20 mL of water and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 27.035 mg of $C_{12}H_{18}N_2O_3S$

Containers and Storage ***Containers***—Well-closed containers.

Tolfenamic Acid



$C_{14}H_{12}ClNO_2$: 261.70

2-(3-Chloro-2-methylanilino)benzoic acid [*13710-19-5*]

Tolfenamic Acid, when dried, contains not less than 99.0 % and not more than 101.0 % of tolfenamic acid ($C_{14}H_{12}ClNO_2$),

Description Tolfenamic Acid is a white or pale yellow, crystalline powder.
Tolfenamic Acid is soluble in *N,N*-dimethylformamide, slightly soluble in ethanol (95) or in dichloromethane, and practically insoluble in water.
Tolfenamic Acid dissolves in dilute sodium hydroxide TS.

Melting point—About 213 °C.

Identification (1) Dissolve 10 mg each of Tolfenamic acid and Tolfenamic acid RS in a mixture of methanol and 1 mol/L hydrochloric acid TS (99 : 1) to make 100 mL, respectively. To 5.0 mL of this solution add a mixture of methanol and 1 mol/L hydrochloric acid TS (99 : 1) to make 50 mL. Determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of solutions of Tolfenamic Acid and Tolfenamic Acid RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Dissolve 25 mg each of Tolfenamic Acid and Tolfenamic acid RS in a mixture of dichloromethane and methanol (3 : 1) to make 10 mL, and use these solutions as the test solution and standard solution, respectively. Perform the test with these solutions as directed under the thin-layer chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for the thin-layer chromatography. Develop the plate with a mixture of toluene, 1,4-dioxane, acetic acid (100) (90 : 25 : 1) to a distance of about 15 cm, air-dry the plate. Examine under ultraviolet light (main wavelength : 254 nm). The principal spots from the test solution and the standard solution show same color and same R_f value.

Purity (1) ***Copper***—Place 1.0 g of Tolfenamic acid in a silica crucible, moisten with sulfuric acid, heat cautiously on a flame for 30 minutes and then heat slowly to about 650 °C. Continue ignition until particles have disappeared. Allow to cool, dissolve the residue in 0.1 mol/L hydrochloric acid TS and dilute to 25.0 mL with the same acid, and use this solution as the test solution. Dilute standard copper stock solution with 0.1 mol/L hydrochloric acid TS to appropriate concentration, and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under Atomic Absorption Spectrophotometry. Determine the concentration of copper in the test solution (10 ppm).

Gas : Dissolved acetylene – Air

Lamp : Copper hollow-cathode lamp
avelength : 324.8 nm

(2) ***Related substances***—Dissolve 50.0 mg of the substance in 5 mL of ethanol (95) and dilute to 50.0 mL with the mobile phase, and use this solution as the test solution. Separately, dissolve 25 mg of Tolfenamic Acid related substance I (2-chlorobenzoic acid) and 25 mg of Tolfenamic Acid related substance II (3-chloro-2-methylaniline) in 5 mL of ethanol (95) and add the mobile phase to make exactly 50 mL, and use this solution as the standard solution (1). Add the mobile phase to 1.0 mL of the test solution, to make exactly 50 mL, add the mobile phase to 1.0 mL of this solution to make 10.0 mL, and use this solution as the standard solution (2). Perform the test with 20 μL each of the test solution and standard solutions (1) and (2) as directed under Liquid Chromatography according to the following operating conditions. The peak area of related substance (1) obtained from the test solution is not more than the peak area of the corresponding peak obtained from standard solution (1) (0.1 %), related substance (2) is not more than half the peak area of the corresponding peak obtained from standard solution (1) (0.05 %). The peak area other than the principal peak from the test solution is not more than the peak area of the principal peak obtained from standard solution (2) (0.1 %), the total area other than the principal peak from the test solution is not more than 5 times the peak areas of the principal peak obtained from the standard solution (2) (0.5 %). The peaks smaller than 0.1 times the peak area of the principal peak obtained from the standard solution (2) are excepted.

Operating conditions
Detector : An Ultraviolet absorption photometer (wavelength: 232 nm)
Column : A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5μm in particle diameter).
Mobile phase : A mixture of ethanol (95), water and acetic acid (100) (650 : 320 : 2).
Flow rate : 0.8 mL/minute
System suitability
System performance : When the procedure is run with the test solution and the standard solution (I) according to the above operating conditions: the relative retention times of related substance I and II to the retention times of tolfenamic acid (about 15 minutes) are 0.25 and 0.34, respectively, with the resolution between the related substance I peak and the related substance II peak being not less than 2.5.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, a constant mass).

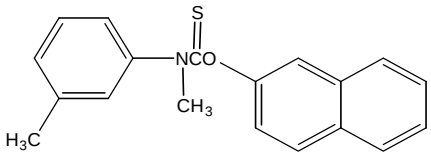
Residue on Drying Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.2 g of Tolfenamic Acid, previously dried, dissolve in 100 mL of ethanol (95), with sonicator and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 0.1 mL of phenol red TS). Perform a blank determination, and make any necessary correction.

1 mL of 0.1 mol/L sodium hydroxide
= 26.171 mg of $C_{14}H_{12}ClNO_2$

Containers and Storage ***Containers***—Well-closed containers.
Storage—Light-resistant.

Tolnaftate



$C_{19}H_{17}NOS$: 307.41

N-Methyl-*N*-(3-methylphenyl)-1-(naphthalen-2-yloxy)methanethioamide [*2398-96-1*]

Tolnaftate, when dried, contains not less than 98.0 % and not more than 101.0 % of tolnaftate ($C_{19}H_{17}NOS$).

Description Tolnaftate appears as white powder and is odorless.
Tolnaftate is freely soluble in chloroform, sparingly soluble in ether, slightly soluble in methanol or in ethanol (95) and practically insoluble in water.

Identification (1) To 0.2 g of Tolnaftate, add 20 mL of potassium hydroxide ethanol TS and 5 mL of water and heat under a reflux condenser for 3 hours. After cooling, to 10 mL of this solution, add 2 mL of acetic acid (100) and shake with 1 mL of lead acetate TS: a black precipitate is produced.
(2) Determine the absorption spectra of solutions of Tolnaftate and Tolnaftate RS in methanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.
(3) Determine the infrared spectra of Tolnaftate and Tolnaftate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 111 ~ 114 °C (after drying).

Purity (1) ***Heavy metals***—Carbonize 1.0 g of Tolnaftate by gentle heating. After cooling, add 5 mL of nitric acid and 1 mL of sulfuric acid and heat until white fumes are evolved. After cooling, add 2 mL of nitric acid and heat until white fumes are evolved. Af-

ter cooling, add 2 mL of nitric acid and 0.5 mL of perchloric acid and heat gradually until white fumes are evolved. Repeat this procedure twice and heat until white fumes are no longer evolved. Incinerate the residue by igniting between 500 °C and 600 °C for 1 hour. Proceed according to Method 2 and perform the test with 50 mL of the test solution so obtained. Prepare the control solution as follows: to 11 mL of nitric acid, add 1 mL of sulfuric acid, 1 mL of perchloric acid and 2 mL of hydrochloric acid, proceed in the same manner as the test solution and add 2.0 mL of standard lead solution and water to make 50 mL (not more than 20 ppm).

(2) ***Related substances***—Dissolve 0.50 g of Tolnaftate in 10 mL of chloroform and use this solution as the test solution. Pipet 2.0 mL of the test solution and add chloroform to make exactly 100 mL. Pipet 5.0 mL of this solution, add chloroform to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with toluene to a distance of about 10 cm and air-dry the plate. Allow the plate to stand in iodine vapor for 5 minutes and examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, in vacuum at a pressure not exceeding 0.67 kPa, 65 °C, 3 hours).

Residue on Ignition Weigh accurately 2.0 g of Tolnaftate and carbonize by gradual heating. Moisten the substance with 1 mL of sulfuric acid, heat gradually until white fumes are no longer evolved and ignite between 450 °C and 550 °C for about 2 hours to constant mass: the residue is not more than 0.1 %.

Assay Weigh accurately about 50 mg each of Tolnaftate and Tolnaftate RS, previously dried, dissolve each in 200 mL of methanol by warming in a water-bath, cool and add methanol to make exactly 250 mL. Pipet 5.0 mL each of the solutions, to each add methanol to make exactly 100 mL and use these solutions as the test solution and the standard solution, respectively. Determine the absorbances, A_T and A_S, of the test solution and the standard solution, respectively, at 257 nm as directed under Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of tolnaftate } (C_{19}H_{17}NOS) = \text{Amount (mg) of Tolnaftate RS} \times \frac{A_T}{A_S}$$

Containers and Storage ***Containers***—Tight containers.

Tolnaftate Cream

Tolnaftate Cream contains not less than 90.0 % and not more than 110.0 % of tolnaftate ($C_{19}H_{17}NOS$: 307.41).

Method of Preparation Prepare as directed under Creams, with Tolnaftate.

Identification Evaporate 10 mL of the next-to-final chloroform solution prepared in the Assay on a steam-bath just to dryness, dissolve the residue in 1 mL of ethanol (95) and use this solution as the test solution. Proceed as directed in the Identification (2) under Tolnaftate.

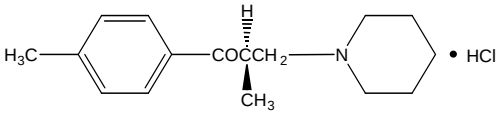
Assay Weigh accurately a portion of Tolnaftate Cream, equivalent to about 10 mg of tolnaftate ($C_{19}H_{17}NOS$) in a separatory funnel and add 75 mL of chloroform. Wash the chloroform solution with 25 mL of 0.1 mol/L sodium hydroxide TS, two 25 mL volumes of 0.1 mol/L hydrochloric acid TS and 25 mL of water. Filter the chloroform layer through a chloroform-washed cotton and dilute with chloroform to make exactly 100 mL. Pipet 5 mL of this solution, dilute with chloroform to make exactly 50 mL and use this solution as the test solution. Separately, weigh accurately a portion of Tolnaftate RS, previously dried (in vacuum, 65 °C, 3 hours), dissolve in chloroform to make solution having a known concentration of about 10 μg per mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution at 258 nm using chloroform as the blank and determine the absorbances, A_T and A_S, for each solution.

$$\text{Amount (mg) of tolnaftate } (C_{19}H_{17}NOS) = C \times \frac{A_T}{A_S}$$

C: Concentration (g/mL) of Tolnaftate RS in the standard solution.

Containers and Storage ***Containers***—Tight containers.

Tolperisone Hydrochloride



and enantiomer

$C_{16}H_{23}NO \cdot HCl$: 281.82

2-Methyl-1-(4-methylphenyl)-3-piperidin-1-ylpropan-1-one hydrochloride [*3644-61-9*]

Tolperisone Hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of tolperisone hydrochloride ($C_{16}H_{23}NO \cdot HCl$).

Description Tolperisone Hydrochloride is a white crystalline powder and has a slight characteristic odor.
Tolperisone Hydrochloride is very soluble in acetic acid (100), freely soluble in water or in ethanol (95), soluble in acetic anhydride, slightly soluble in acetone, and practically insoluble in ether.
Tolperisone Hydrochloride is hygroscopic.

pH—Dissolve 1.0 g of Tolperisone Hydrochloride in 20 mL of water: the pH of this solution is between 4.5 and 5.5.

Melting point—167 ~ 174 °C.

Identification (1) Dissolve 0.2 g of Tolperisone Hydrochloride in 2 mL of ethanol (95), add 2 mL of 1,3-dinitrobenzene TS and 2 mL of sodium hydroxide TS and heat: a red color is observed.

(2) To 5 mL of a solution of Tolperisone Hydrochloride (1 in 20), add 2 to 3 drops of iodine TS: a red-brown precipitate is produced.

(3) Dissolve 0.5 g of Tolperisone Hydrochloride in 5 mL of water, add 2 mL of ammonium TS and filter. Acidify 5 mL of the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests for chloride.

Absorbance $E_{1cm}^{1\%}$ (257 nm): 555 ~ 585 (after drying, 5 mg, ethanol (95), 500 mL).

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Tolperisone Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) ***Sulfate***—Perform the test using 4.0 g of Tolperisone Hydrochloride. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.005 %).

(3) ***Heavy metals***—Proceed with 1.0 g of Tolperisone Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Piperidine hydrochloride***—Dissolve 0.20 g of Tolperisone Hydrochloride in water to make exactly 10 mL and use this solution as the test solution. Separately, dissolve 20 mg of Piperidine Hydrochloride RS in water to make exactly 1000 mL and use this solution as the standard solution. Transfer exactly 5 mL each of the test solution and the standard solution to different separatory funnels, add 0.1 mL each of a solution of cupric sulfate (1 in 20), then add 0.1 mL each of ammonia solution (28) and exactly 10 mL each of a mixture of isooctane and carbon disulfide (3 : 1) and shake vigorously for 30 minutes. Immediately after allowing to stand, separate the isooctane-carbon disulfide mixture layer and dry with anhydrous sodium sulfate. Perform the test with the test solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry: the absorbance of the test solution at 438 nm is not more than that of the standard solution.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, silica gel, 3 hours).

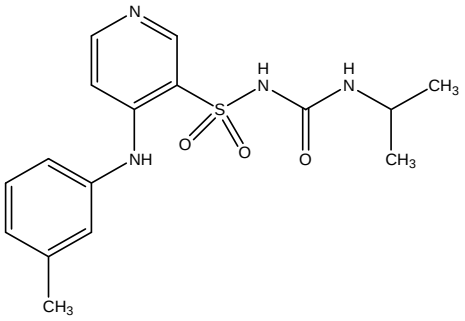
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.5g of Tolperisone Hydrochloride, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 28.182 mg of $C_{16}H_{23}NO \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Torsemide



$C_{16}H_{20}N_4O_3S$: 348.42

1-[4-(3-Methylanilino)pyridin-3-yl]sulfonyl-3-propan-2-ylurea [*56211-40-6*]

Torsemide contains not less than 98.0 % and not more than 102.0 % of torsemide ($C_{16}H_{20}N_4O_3S$), calculated on the anhydrous basis.

Description Torsemide is a white, crystalline powder.
Torsemide is slightly soluble in ethanol (95) or in methanol, very slightly soluble in acetone or in chloroform, and practically insoluble in water or in ether.

Identification (1) Determine the infrared spectra of Torsemide and Torsemide RS, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) The retention time of the principal peak obtained from the test solution in the Assay corresponds to the retention time of the principal peak obtained from the standard solution.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Torsemide according to Method 2 and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Use the test solution obtained in the Assay as the test solution. Separately, weigh accurately about 8 mg each of Torsemide related substance I, {4-[(3-methylphenyl) amino]-3-pyridinesulfonamide} RS, Torsemide related Compound II {*N*-[(*n*-butylamino)carbonyl]-4-[(3-methylphenyl)amino]-3-pyridinesulfoneamide} RS and Torsemide related Compound III, {*N*-[ethylamino)carbonyl]-4-[(3-methyl-phenyl)amino]-3-pyridinesulfoneamide} RS, add 30 mL of methanol, sonicate to dissolve for not less than 8 minutes. Add 45 mL of 0.02 mol/L potassium phosphate buffer, cool to room temperature, dilute with mobile phase to make exactly 100 mL. Quantitatively dilute a portion of this solution with mobile phase to obtain a solution having a known concentration of about 0.0019 mg per mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions in the Assay, measure the peak areas of the related substance I, the related substance II and the related substance III in each solution by using the automatic integration method, calculate the amount (mg) of the related substances by equation 1: the relative substance I is not more than 0.5 %, the related substance II is not more than 0.3 %, the relative substance III is not more than 0.2 %. Calculate the each amount (mg) of any other related substances by equation (2). The value is not more than 0.1 %, the sum of amount (mg) of these substances is not more than 0.2 %, and the amount of total related substances containing the related substance I, the related substance II and the related substance III is not more than 1.0 %.

$$\text{Amount (mg) of related substances} = 100 \times \frac{C_S}{C_T} \times \frac{A_T}{A_S} \qquad (1)$$

C_S: Concentration (mg/mL) of each the related substance in the standard solution.

C_T: Concentration (mg/mL) of Torsemide in the test solution.

A_S: Peak area of each the related substance obtained from the standard solution.

A_T: Peak area of each the related substance obtained from the test solution.

$$\text{Any other related substances (mg)} = 100 \times \frac{A_i}{A_S} \qquad (2)$$

A_i: Peak area other than the related substances I, II and III obtained from the test solution.

A_S: Sum of peak areas of total peaks obtained from the test solution.

System suitability

System performance : Weigh about 3 mg each of Torsemide RS and Torsemide related substance I RS, dissolve in 3 mL of methanol, with sonication for not less than 8 minutes. add 4.5 mL of 0.02 mol/L Potassium dihydrogen phosphate bufffer, cool to room temperature, dilute with mobile phase to make exactly 10 mL. When the procedure is run with 20 μL of this solution according to the operating conditions as directed in the Assay, the resolution of peaks between torsemide and torsemide related substance I is not less than 1.0, and the symmetry factor is not more than 2.0.

System repeatability : When the test is repeated 6 times with 20 μL each of standard solution according to the operating conditions as directed under the Assay, the relative standard deviation of the peak area of Torsemide is not more than 10.0 %.

Water Not more than 1.0 % (1 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 38 mg of Torsemide, add 30 mL of methanol, and dissolve with sonication for not less than 8 minutes. Add 45 mL of 0.02 mol/L potassium dihydrogen phosphate buffer, dilute with mobile phase to make exactly 100 mL. and use this solution as the test solution. Separately, weigh accurately about 19 mg of Torsemide RS, dissolve in 15 mL of methanol, with sonication for not less than 8 minutes. Add 22.5 mL of 0.02 mol/L potassium dihydrogen phosphate buffer, dilute with mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with each 20 μL of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, measure the peak areas of torsemide obtained from each solutions, A_T and A_S.

$$\text{Amount (mg) of torsemide } (C_{16}H_{20}N_4O_3S) = 100 \times C \times \frac{A_T}{A_S}$$

C: Concentration (mg/mL) of Torsemide in the standard solution.

Operation conditions

Detector : An ultraviolet absorption photometer (wavelength: 288 nm).

Column : A stainless steel column, about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Mobile phase : A mixture of 0.02 mol/L potassium dihydrogen phosphate buffer solution and methanol (3:2).

Flow rate : 1.5 mL/minute.

System suitability

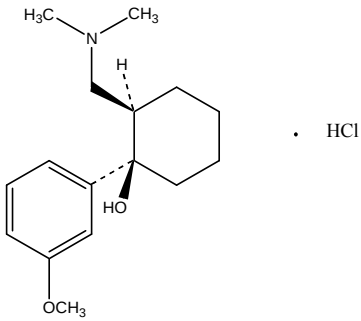
System performance : When the procedure is run with 20 μL of the standard solution under the above operating conditions, the symmetry factor of the peak of torsemide is not more than 2.0.

System repeatability : When the test is repeated 5 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of torsemide is not more than 2.0 %.

0.02 mol/L Potassium phosphate buffer—Dissolve 2.7 g of potassium dihydrogen phosphate in about 900 mL of water. Adjust with phosphoric acid to pH of 3.5, and dilute with water to make 1000mL.

Containers and Storage ***Containers***—Well-closed containers.

Tramadol Hydrochloride



· HCl

and enantiomer

$C_{16}H_{25}NO_2 \cdot HCl$: 299.84

(1*R*,2*R*)-2-[(Dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexan-1-ol hydrochloride [*36282-47-0*]

Tramadol Hydrochloride contains not less than 99.0 % and not more than 101.0 % of tramadol hydrochloride ($C_{16}H_{25}NO_2 \cdot HCl$), calculated on the anhydrous basis.

Description Tramadol Hydrochloride is a white, crystalline powder.
Tramadol Hydrochloride is very soluble in water or in methanol, and practically insoluble in acetone.

Identification (1) Determine the infrared spectra of Tramadol Hydrochloride and Tramadol Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Perform the test with, as directed under Thin-layer Chromatography for the Related Substance I in Purity test as directed under Thin-layer Chromatography: the spot from the test solution (2) shows the same R_f value as the spot from the test solution (1)

(3) A solution of Tramadol Hydrochloride (1 in 50) responds to the Qualitative Test (2) for chlorides.

Melting Point 180 ~ 184 °C.

Specific Optical Rotation $[\alpha]_D^{20}$: -0.10 ~ +0.10° (1.0 g, water, 20 mL, 100 mm)

Purity (1) ***Clarty and color of solution***—Dissolve 1.0 g of Tramadol Hydrochloride in 20 mL of water: the solution is colorless and clear.

(2) ***Acidity***—Dissolve 1.0 g of Tramadol Hydrochloride in 20 mL of water, add 0.1 mL of methyl red TS to 10 mL of this solution, add 0.2 mL of 0.01 mol/L hydrochloric acid VS: a red color is produced. Not more than 0.4 mL of 0.01 mol/L sodium hydroxide is required to change the color of the solution to yellow.

(3) ***Heavy metals***—Proceed with 1.0 g of Tramadol Hydrochloride according to the method 1, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Related substance I***—Weigh 0.10 g of Tramadol Hydrochloride, dissolve in 2 mL of methanol, and use this solution as the test solution (1). Pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the test solution (2). Separately, weigh 50 mg of Tramadol Hydrochloride RS, dissolve in methanol to make 10 mL, and use this solution as the standard solution (1). Weigh 10 mg of Tramadol Hydrochloride related substance I RS {(2*RS*)-2-[(dimethylamino)methyl]cyclo-hexanone}, dissolve in 10 mL of methanol, pipet 1 mL of this solution, add methanol to make 10 mL, and use this solution as the standard solution (2). Weigh 5 mg of Tramadol Hydrochloride related substance II RS {(1*RS*,2*RS*)-2-[(dimethylamino)methyl]-1-(3-methoxy-phenyl) cyclohexanol}, dissolve in 1 mL of the standard solution (1), and use this solution as the standard solution (3). Perform the test with the test solution and the standard solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and standard solutions (2) and (3) on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop not less than 2/3 of the plate, and saturate the plate for 20 minutes with ammonia solution (28). For this, add ammonia solution (28) to one trough of a twin trough tank. Just before developing, add a mixture of toluene, 2-propanol, and strong ammonia water (80 : 19 : 1) to the distance of about 10 cm, air-dry the plate. Place the plate in the chromatographic tank, ensuring that the layer of silica gel is oriented towards the middle of the tank. Expose the plate to iodine vapour for 1 hour, examine under ultraviolet light (main wavelength: 254 nm). The spot of the related substance I obtained from test solution (1) is not more intense and not greater than the spot obtained from standard solution (2) (0.2 %). When 2 spots in the chromatogram obtained from the standard solution (3) are crearly separated, it is effective.

(5) ***Related substance II***—Weigh 0.15 g of Tramadol Hydrochloride, dissolve in the mobile phase to

make exactly 100 mL, and use this solution as the test solution. Pipet 2.0 mL of the test solution, add the mobile phase to make 10 mL, pipet 1.0 mL of this solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with each 20 μL of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and measure the peak areas of each solution according to the automatic integration method. The peak area of the related substance II obtained from the test solution is not greater than 0.5 times the principal peak area of obtained from the standard solution (0.2 %), the peak area other than the principal peak is not more than 0.5 times the principal peak obtained from the standard solution (0.1 %), the total area of peaks other than the principal peak is not more than 2 times the peak area of the principal peak obtained from the standard solution (0.4 %). The peaks less than 0.1 times the peak area of the principal peak obtained from the standard solution are excepted.

Operating conditions

Detector : An Ultraviolet absorption photometer (wavelength: 270 nm)

Column: A stainless steel column, about 4 mm in internal diameter and about 25 cm in length, packed with base-deactivated octadecylsilanized silica gel for the Liquid Chromatography (5 μm in particle diameter).

Mobile phase : A mixture of a mixture of trifluoroacetic acid and water (100 : 0.2), and acetonitrile (705 : 295)

Flow rate : 1.0 mL/minute

System suitability

System performance : Dissolve 5 mg of Tramadol related substance II RS in 4.0 mL of the test solution, add the mobile phase to make 100 mL. When the procedure is run with 20 μL of this solution, as directed under the above operating conditions, the resolution between tramadol related substance II peak and tramadol peak is not less than 2.0.

Water Not more than 0.5 % (1 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

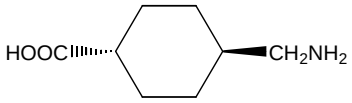
Assay Weigh accurately about 0.18 g of Tramadol Hydrocholride, dissolve in 25 mL of acetic acid (100), add 10 mL of acetic anhydride, mix, and titrate with 0.1 mol/L perchloric acid (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

1 mL of 0.1 mol/L perchloric acid
= 29.984 mg of $C_{16}H_{25}NO_2 \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Tranexamic Acid



$C_8H_{15}NO_2$: 157.21

4-(Aminomethyl)cyclohexane-1-carboxylic acid
[*1197-18-8*]

Tranexamic Acid, when dried, contains not lest than 99.0 % and not more than 101.0 % of tranexamic Acid ($C_8H_{15}NO_2$).

Description Tranexamic Acid appears as white crystals or crystalline powder, is odorless and has a bitter taste.
Tranexamic Acid is freely soluble in water, and practically insoluble in ethanol (99.5).
Tranexamic Acid dissolves in sodium hydroxide TS.

Identification Determine the infrared spectra of Tranexamic Acid and Tranexamic Acid RS, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit the similar intensities of absorption at the same wavenumbers.

pH Dissolve 1.0 g of Tranexamic Acid in 20 mL of water: the pH of this solution is between 7.0 and 8.0.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Tranexamic Acid in 10 mL of water: the solution is clear and colorless.

(2) ***Chloride***—Perform the test with 1.0 g of Tranexamic Acid. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014 %).

(3) ***Heavy metals***—Dissolve 2.0 g of Tranexamic Acid in water to make 20 mL, and use this solution as the test stock solution. To 12 mL of the test stock solution add 2 mL of hydrochloric acid-ammonium acetate butler solulion. pH 3.5, mix, add 1.2 mL of thioacetamide TS, mix immediately, and use this solution as tle test solution. Separately, proceed in the same manner as above with a mixture of 1 mL of standard lead solution, 2 mL of the test stock solution and 9 mL of water, and use the solution so obtained as the standard solution. Separately, proceed in the same manner with a mixture of 10 mL of water and 2 mL of the test stock solution, and use the solution so obtained as the control solution. Conform that the color of the standard solution is slightly darker than that of the control solution. Compare the test solution and the standard solution 2 minutes after they are prepared: the color of the test solution is not more intense than that of the standard solution (not more than 10 ppm).

(4) ***Arsenic***—Prepare the test solution with 1.0 g of Tranexamic Acid according to Method 1 and perform the test (not more than 2 ppm).

(5) ***Related substances***—Dissolve 0.20 g of Tranexamic Acid in water to make exactly 20 mL, and use this solution as the test solution. Pipet 5 mL of the test solution, and add water to make exactly 100 mL. Pipet 1 ml of this solution, add water to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the test solution and test standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area multiplied by relative response factor 1.2 of the peak, having the relative retention time of about 1.5 with respect to tranexarnic acid, is not more than 2/5 of the peak area of tranexamic acid from the standard solution, and the area of the peak, having the relative retention time of about 2.1 with respect to tranexamic acid, is not more than 1/5 of the peak area of tranexamic acid from the standard solution. The area of each peak other than tranexamic acid and other than the peaks mentioned above is not more than 1/5 of the peak area of tranexamic acid from the standard solution. For this comparison, use the areas of the peaks, having the relative retention time of about 1.1 and about 1.3, after multiplying by their relative response factors 0.005 and 0.006, respectively. The total area of the peaks other than tranexamic acid is not more than the peak area of tranexamic acid from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability

System performance: Proceed as directed in the System suitability under the Assay.

Test for required detectability: To exactly 5 mL of the standard solution add water to make exactly 25 mL. Confirm that the peak area of tranexamic acid obtained from 20 μL of this solution is equivalent to 14 to 26 % of that from 20 μL of the standard solution.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tranexamic acid is not more than 7 %.

Time span of measurement: About 3 times as long as the retention time of tranexamic acid beginning after the solvent peak.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 50 mg each of Tranexamic Acid and Tranexamnic Acid RS, previously dried, dissolve in water to make exactly 25 mL, and use these solutions as the test solution and standard solution. Perform the test with exactly 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of tranexamic acid from each solution.

$$\text{Amount (mg) of tranexamic acid } (C_8H_{15}NO_2) = \text{Amount (mg) of Tranexamic Acid RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 6.0 mm in internal diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: Dissolve 11.0 g of sodium dihydrogen phosphate in 500 mL of water, and add 5 mL of triethylamine and 1.4 g of sodium lauryl sulfate. Adjust the pH to 2.5 with phosphoric acid or diluted phosphoric acid (1 in 10), add water to make 600 mL, and add 400 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of tranexamic acid is about 20 minutes.

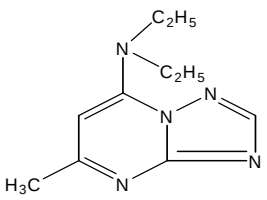
System suitability

System performance: To 5 mL of the standard solution add 1 mL of a solution of 4-(aminomethyl) benzoic acid (1 in 10,000) and water to make 50 mL. when the procedure is run with 20 μL of this solution under the above operating condition,, tranexamic acid and 4-(aminomethyl)benzoic acid are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution tinder the above operating conditions, the relative standard deviation of the peak area of tranexamic acid is not more than 0.6 %.

Containers and Storage ***Containers***—Well-closed containers.

Trapidil



$C_{10}H_{15}N_5$: 205.26

N,N-Diethyl-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-7-amine [*15421-84-8*]

Trapidil, when dried, contains not less than 98.5 % and not more than 101.0 % of trapidil ($C_{10}H_{15}N_5$).

Description Trapidil apears as white to pale yellowish white, crystalline powder.
Trapidil is very soluble in water or in methanol, freely soluble in ethanol (95), in acetic anhydride and in acetic acid (100) and sparingly soluble in ether.

pH—Dissolve 1.0 g of Trapidil in 100 mL of water: the pH of this solution is between 6.5 and 7.5.

Identification (1) To 5 mL of a solution of Trapidil (1 in 50), add 3 drops of Dragendorff's TS: an orange color is observed.

(2) Determine the absorption spectra of solutions of Trapidil and Trapidil RS (1 in 125000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit the similar intensities of absorption at the same wavelengths.

Melting Point 101 ~ 105 °C.

Absorbance $E^{1\%}_{1cm}$ (307 nm): 860 ~ 892 (after drying, 20 mg, water, 2500 mL).

Purity (1) ***Clarity and color of solution***—Dissolve 2.5 g of Trapidil in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) ***Chloride***—Perform the test with 0.5 g of Trapidil. Prepare the control solution with 0.25 mL of 0.01 mo1/L hydrochloric acid VS (not more than 0.018 %).

(3) ***Ammonium***—Place 50 mg of Trapidil in a glass-stoppered Erlenmeyer flask, thoroughly moisten with 10 drops of sodium hydroxide TS and stopper the flask. Allow it to stand at 37 °C for 15 minutes: the gas evolved does not change moistened red litmus paper to blue.

(4) ***Heavy metals***—Dissolve 1.0 g of Trapidil in 40 mL of water and add 1.5 mL of dilute hydrochloric acid, 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 1.0 mL of standard lead solution, add 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(5) ***Arsenic***—Prepare the test solution with 1.0 g of Trapidil according to Method 1 and perform the test (not more than 2 ppm).

(6) ***Related substances***—Dissolve 0.10 g of Trapidil in 4 mL of methanol and use this solution as the test solution. Pipet 1 mL of the test solution and add methanol to make exactly 20 mL. Pipet 1 mL of this solution, add methanol to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 20 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, ethanol (95) and acetic acid (100) (85 : 13 : 2) to a distance of about 10 cm and air-dry the plate. Allow the plate to stand in iodine vapor for 60 minutes: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, silica gel, 60 °C, 3 hours).

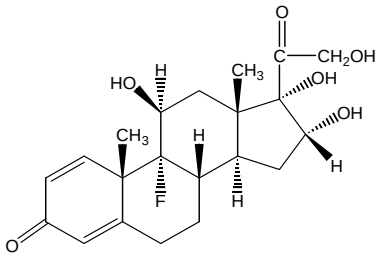
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.2 g of Trapidil, previously dried, dissolve in 20 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L percholic acid VS
=20.526 mg of $C_{10}H_{15}N_5$

Containers and Storage ***Containers***—Tight containers.

Triamcinolone



$C_{21}H_{27}FO_6$: 394.43

(1*R*,2*S*,10*S*,11*S*,13*R*,14*S*,15*S*,17*S*)-1-Fluoro-13,14,17-trihydroxy-14-(2-hydroxyacetyl)-2,15-dimethyltetracyclo[8.7.0.0^{2,7}.0^{11,15}] heptadeca-3,6-dien-5-one [*124-94-7*]

Triamcinolone, when dried, contains not less than 97.0 % and not more than 103.0 % of triamcinolone ($C_{21}H_{27}FO_6$).

Description Triamcinolone appears as white, crystalline powder and is odorless.
Triamcinone is freely soluble in *N,N*-dimethylformamide, slightly soluble in methanol, in ethanol (95) or in acetone, and practically insoluble in water, in 2-propanol or in ether.

Melting point—About 264 °C (with decomposition).

Identification (1) Dissolve 1 mg of Triamcinolone in 6 mL of ethanol (95), add 5 mL of 2,6-di-*t*-butylcresol TS and 5 mL of sodium hydroxide TS and heat in a water-bath for 30 minutes under a reflux condenser: a red-purple color is observed.

(2) Add 5 mL of water and 1 mL of Fehling's TS to 10 mg of Triamcinolone and heat: a red precipitate is produced.

(3) Proceed with 10 mg of Triamcinolone as directed under Oxygen Flask Combustion Method, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid. When combustion is completed, shake vigorously so as to absorb the gas evolved: the solution responds to the Qualitative Tests for fluoride.

(4) Determine the infrared spectra of Triamcinolone and Triamcinolone RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit the similar intensities of absorption at the same wavenumbers. If any differences appear, dissolve 0.1 g each of Triamcinolone and Triamcinolone RS in 7 mL of a mixture of 2-propanol and water (2 : 1) by warming. Cool the solutions in ice-water, filter the crystals produced, wash with two 10 mL volumes of water, dry and repeat the test on the residues.

Specific Optical Rotation $[\alpha]_D^{20}$: +65 ~ +71° (0.1 g after drying, *N,N*-dimethylformamide, 10 mL, 100 mm).

Purity ***Heavy metals***—Proceed with 0.5 g of Triamcinolone according to Method 2 and perform the test. Prepare the control solution with 1.5 mL of standard lead solution (not more than 30 ppm).

Loss on drying Not more than 2.0 % (0.5 g, in vacuum, P_2O_5, 60 °C, 3 hours).

Residue on Ignition Not more than 0.3 % (0.5 g, platinum crucible).

Assay Dissolve about 20 mg each of Triamcinolone and Triamcinolone RS, previously dried and accurately weighed, in a solution of L-ascorbic acid in methanol (1 in 1000) to make exactly 50 mL. Pipet 5 mL each of these solutions, add 5 mL each of the internal standard solution, add a solution of L-ascorbic acid in methanol (1 in 1000) to make exactly 20 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 µL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak height of triamcinolone to that of the internal standard, for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of triamcinolone } (C_{21}H_{27}FO_6) = \text{Amount (mg) of Triamcinolone RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—Dissolve 15 mg of methyl parahydroxybenzoate in a solution of L-ascorbic acid in methanol (1 in 1000) to make 100 mL.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and 15 to 30 cm in length, packed with octadecylsilanized silica gel (5 to 10 µm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: A mixture of water and acetonitrile (3 : 1).

Flow rate: Adjust the flow rate so that the retention time of triamcinolone is about 10 minutes.

System suitability

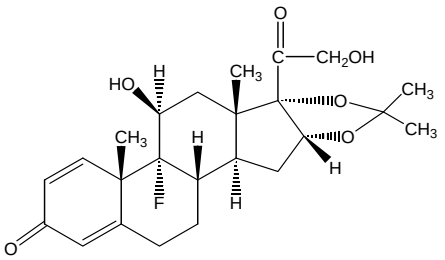
System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, triamcinolone and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 µL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of triamcinolone to that of the internal standard is not more than 1.5 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Triamcinolone Acetonide



$C_{24}H_{31}FO_6$: 434.50

(4a*S*,4b*R*,5*S*,6a*S*,6b*S*,9a*R*,10a*S*,10b*S*)-4b-Fluoro-6b-glycoloyl-5-hydroxy-4a,6a,8,8-tetramethyl-4a,4b,5,6,6a,6b,9a,10,10a,10b,11,12-dodecahydro-2*H*-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-2-one [*76-25-5*]

Triamcinolone Acetonide, when dried, contains not less than 97.0 % and not more than 103.0 % of triamcinolone acetonide ($C_{24}H_{31}FO_6$).

Description Triamcinolone Acetonide is a white, crystalline powder and is odorless.
Triamcinolone Acetonide is sparingly soluble in ethanol (99.5), in acetone or in 1,4-dioxane, slightly soluble in methanol or in ethanol (95), and practically insoluble in water or in ether.

Melting point—About 290 °C (with decomposition).

Identification (1) Dissolve 2 mg of Triamcinolone Acetonide in 40 mL of ethanol (95), add 5 mL of 2,6-di-*t*-butylcresol TS and 5 mL of sodium hydroxide TS and heat in a water-bath under a reflux condenser for 20 minutes: a green color is observed.

(2) Add 5 mL of water and 1 mL of Fehling's TS to 10 mg of Triamcinolone Acetonide and heat: a red precipitate is produced.

(3) Proceed with 10 mg of Triamcinolone Acetonide as directed under Oxygen Flask Combustion Method, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid. When combustion is completed, shake vigorously so as to absorb the gas evolved: the solution responds to the Qualitative Tests for fluoride.

(4) Determine the ultraviolet absorption spectra of solutions of Triamcinolone Acetonide and Triamcinolone Acetonide RS in ethanol (95) (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(5) Determine the infrared spectra of Triamcinolone Acetonide and Triamcinolone Acetonide RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers. If any differences appear, dissolve 0.1 g each of Triamcinolone Acetonide and Triamcinolone Acetonide RS in 20 mL of ethanol (95), respectively, evaporate to dryness and repeat the test on the residues.

Specific Optical Rotation $[\alpha]_D^{20}$: +118 ~ +130° (0.05 g, *N,N*-dimethylformamide, 10 mL, 100 mm).

Purity (1) ***Heavy metals***—Proceed with 0.5 g of Triamcinolone Acetonide according to Method 2 and perform the test. Prepare the control solution with 1.5 mL of standard lead solution (not more than 30 ppm).

(2) ***Related substances***—Dissolve 40 mg of Triamcinolone Acetonide in 4 mL of acetone and use this solution as the test solution. Pipet 1 mL of the test solution, add acetone to make exactly 100 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 20 μL each of the test solution and the standard solution on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (93 : 7) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 2.0 % (0.5 g, in vacuum, P_2O_5, 60 °C, 3 hours).

Residue on Ignition Not more than 0.2 % (0.5 g, platinum crucible).

Assay Dissolve about 20 mg each of Triamcinolone Acetonide and Triamcinolone Acetonide RS, previously dried and accurately weighed, in methanol to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 10 mL each of the internal standard solution, then add the mobile phase to make exactly 50 mL and use these solutions as the test solution and the standard solution, respectively. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak height of triamcinolone acetonide to that of the internal standard, respectively for the test solution and the standard solution.

$$\text{Amount (mg) of triamcinolone acetonide } (C_{24}H_{31}FO_6) = \text{Amount (mg) of Triamcinolone Acetonide RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of prednisolone in methanol (1 in 5000).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and 15 to 30 cm in length, packed with octadecylsilanized silica gel (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: A mixture of water and acetonitrile (3 : 1).

Flow rate: Adjust the flow rate so that the retention time of triamcinolone acetonide is about 13 minutes.

System suitability

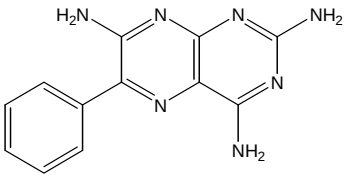
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and triamcinolone acetonide are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL each of standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of triamcinolone acetonide to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Triamterene



$C_{12}H_{11}N_7$: 253.26

6-Phenylpteridine-2,4,7-triamine [*396-01-0*]

Triamterene, when dried, contains not less than 98.5 % and not more than 101.0 % of triamterene ($C_{12}H_{11}N_7$).

Description Triamterene is a yellow, crystalline powder, is odorless and tasteless.
Triamterene is sparingly soluble in dimethylsulfoxide, very slightly soluble in acetic acid (100), and practically insoluble in water, in ethanol (95) or in ether.
Triamterene dissolves in nitric acid or in sulfuric acid, but does not dissolve in dilute nitric acid, in dilute sulfuric acid or in dilute hydrochloric acid.

Identification (1) To 10 mg of Triamterene, add 10 mL of water, heat and filter after cooling: the filtrate shows a purple fluorescence. To 2 mL of the filtrate, add 0.5 mL of hydrochloric acid: the fluorescence disappears.

(2) The filtrate obtained in (1) responds to the Qualitative Tests for primary aromatic amines.

(3) Dissolve 10 mg each of Triameterene and Triameterene RS in 100 mL of acetic acid (100). To 10 mL each of these solutions, add water to make 100 mL. Determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Triamterene according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Arsenic***—Prepare the test solution with 1.0 g of Triamterene according to Method 3 and perform the test (not more than 2 ppm).

(3) ***Related substances***—Dissolve 0.10 g of Triamterene in 20 mL of dimethylsulfoxide. Pipet 2 mL of this solution, add methanol to make exactly 50 mL and use this solution as the test solution. Pipet 1 mL of the test solution, add methanol to make exactly 200 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, strong ammonia water and methanol (9 : 1 : 1) to a distance of about 10 cm and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 4 hours).

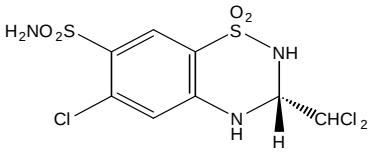
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.15 g of Triamterene, previously dried and dissolve in 100 mL of acetic acid (100) by warming, cool and titrate with 0.05 mol/L perchloric acid VS (indicator: 2 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 12.663 mg of $C_{12}H_{11}N_7$

Containers and Storage ***Containers***—Well-closed containers.

Trichlormethiazide



and enantiomer

$C_8H_8Cl_3N_3O_4S_2$: 380.66

6-Chloro-3-(dichloromethyl)-1,1-dioxo-3,4-dihydro-2*H*-1λ6,2,4-benzothiadiazine-7-sulfonamide [*133-67-5*]

Trichlormethiazide, when dried, contains not less than 97.5 % and not more than 102.0 % of trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$).

Description Trichlormethiazide appears as white powder.
Trichlormethiazide is freely soluble in *N,N*-dimethylformamide or in acetone, slightly soluble in in acetonitrile or in ethanol (95), and practically insoluble in water.
A Solution of Trichlormethiazide in aceton (1 in 50) shows no optical rotation.

Melting point—About 270 °C (with decomposition).

Identification (1) Determine the absorption spectra of solutions of Trichiormethiazide and Trichiormethiazide RS in ethanol (95) (3 in 250000) as

directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Trichlormethiazide and Trichlormethiazide RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Perform the test with Trichiormethiazide as directed under the Flame Coloration Test (2): a green color appears.

Purity (1) ***Chloride***—Dissolve 1.0 g of Trichlormethiazide in 30 mL of acetone, add 6 mL of dilute nitric acid and water to make 50 mL and perform the test using this solulion as the test solution. Prepare the control solution as follows: to 1.0 mL of 0.01 mol/L hydrochloric acid VS, add 30 mL of acetone, 6 mL of dilute nitiric acid and water to make 50 mL (not more than 0.036 %).

(2) ***Sulfate***—Dissolve 1.0 g of Trichlormethiaside in 30 mL of acetone, add 1 mL of dilute hydrochloric acid and water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution as follows: to 1.0 mL of 0.005 mol/L sulfuric acid VS, add 30 mL of acetone, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048 %).

(3) ***Heavy metals***—Proceed with 1.0 g of Trichlormethiazide according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Selenium***—Proceed with 0.2 g of Trichlormethiazide as directed under Oxygen Flask Combustion Method, using 25 mL of diluted nitric acid (1 in 30) as the absorbing liquid. Use a 1 L combustion flask. After combustion, wash the stopper and the inside of the flask with 10 mL of water. Transfer the liquid inside the flask to a 150 mL beaker, using about 20 mL of water. Heat gently to boil, boil for 10 minutes, cool to room temperature, and use this solution as the test solution. Separately, pipet 6.0 mL of selenium standard stock solution, add 25 mL of diluted nitric acid (1 in 30) and 25 mL of water, and use this solution as the standard solution. Adjust the pH of the test solution and standard solution to 2.0 ± 0.2 with diluted ammonia solution (28) (1 in 2), add water to make exactly 60 mL, transfer to a separatory funnel using 10 mL of water, and wash the separatory funnel with 10 mL of water. Add 0.2 g of hydroxylamine hydrochloride, stir to dissolve, add immediately 5.0 mL of 2,3-diamino-naphthalene TS, stopper, stir to mix, and allow to stand at room temperature for 100 minutes. Add 5.0 mL of cyclohexane, shake vigorously for 2 minutes, allow the layers to separate, discard the water layer, centrifuge the cyclohexane extract to remove water, and take the cyclohexane layer. Determine the absorbances at about 380 nm of these solutions as directed under Ultraviolet-visible Spectrophotometry, using a solution prepared by adding 25 mL of water to 25 mL of diluted nitric acid (1 in 30) and proceeding in the same manner, as the blank: the absorbance obtained from the test solution is not more than that from the standard solution (not more than 30 ppm).

(5) ***Arsenic***—Prepare the test solution with 0.6 g of Trichlormethiazide according to Method 3 and perform the test. Use 20 mL of *N,N*-dimethylformamide (not more than 3.3 ppm).

(6) ***Related substances***—Dissolve 25 mg of Trichlormethiazide in 50 mL of acetoiihrile, and use this solution as the test solution. Perform the test with 10 μL of the test solution as directed under Liquid Chromatography according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of related substances by the area percentage method: the amount of 4-amino-6-chloro-benzene-l,3-disulfonamide, having the relative retention time of about 0.3 with respect to trichlormethiazide, is not more than 2.0 %, and the total amount of the related substances is not more than 2.5 %.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 268 nmn).

Column: A stainless steel column 4.6 mm in internal diameter and 15 cm in length, packed with phenylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Control the gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3:1).

Mobile phase B: A mixture of acetonitrile and diluted phosphoric acid (1 in 1000) (3:1).

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-10	100	0
10-20	100→0	0→100

Flow rate: 1.5 mL per minute

System suitability

Test for required detectability: To exactly 1 mL of the test solution, add acctonitrile to make exactly 50 rnL, and use this solution as the system suitability solution. Pipet 1 mL of the system suitability solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of trichlormethiazide obtained from 10 μL of this solution is equivalent to 3.5 to 6.5 % of that of trichlormethiazide obtained from 10 μL of the system suitability solution.

System performance: To 5 mL of the system suitability solution add 5 mL of water, and warm in a water bath at 60 °C for 30 minutes. When the procedure is run with 10 μL of this solution, after cooling, under the above operating conditions, 4-amino-6-

chlorobenzene-1,3-disulfonamide and trichlormethiazide are eluted in this order, the relative retention time of 4-amino-6-chlorobenzene-1,3-disulfonamide with respect to trichlormethiazide is about 0.3, and the number of theoretical plates and the symmetry factor of the peak of trichlormethiazide are not less than 5000 and not more than 1.2, respectively.

System repeatability: When the test is repeated 3 times with 10 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the peak area of trichlormethiazide is not more than 2.0 %.

Time span of measurement: About 2.5 times as long as the retention time of trichlormethiazide beginning after the solvent peak.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours)

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 25 mg each of Trichlormethiazide and Trichlormethiazide RS, previously dried, and dissolve in exactly 20 mL of the internal standard solution. To exactly 1 mL each of these solutions add acetonitrile to make exactly 20 mL, and use these solutions as the test solution and the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S of the peak area of trichlormethiazide to that of the internal standard for the test solution and the standard solution.

$$\text{Amount (mg) of trichlormethiazide } (C_8H_8Cl_3N_3O_4S_2) = \text{Amount (mg) of Trichlormethiazide RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of 3-nitrophenol in acetonitrile (1 in 800).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 268 nm).

Column: A stainless steel column 4.6 mm in internal diameter and 15 cm in length, packed with phenylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3 : 1).

Flow rate: Adjust the flow rate so that the retention time of trichlormethiazide is about 8 minutcs.

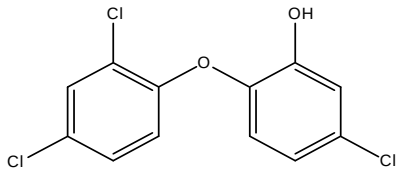
System suitability

System performance: When the procedure is run with l0 μL of the standard solution under the above operating conditions, the internal standard and trichlormethiazide are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of trichlormethiazide to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Well-closed containers.

Triclosan



$C_{12}H_7Cl_3O_2$: 289.54

5-Chloro-2-(2,4-dichlorophenoxy)phenol [*3380-34-5*]

Triclosan contains not less than 97.0 % and not more than 103.0 % of triclosan ($C_{12}H_7Cl_3O_2$), calculated on the anhydrous basis.

Description Triclosan is a white, crystalline powder. Triclosan is very soluble in methanol, in ethanol (95) or in acetone, slightly soluble in hexane, and practically insoluble in water.

Melting point—about 57 °C.

Identification (1) Determine the infrared spectra of Triclosan and Triclosan RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) The retention time of the principal peak obtained from the test solution corresponds to the retention time of the principal peak obtained from the standard solution.

Purity (1) ***Clarity of solution***—Dissolve 1.40 g of Triclosan in 10 mL of acetone: the solution is clear.

(2) ***Heavy metals***—Proceed with 1.0 g of Triclosan according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(3) ***Related substances***—Perform the test with about 0.5 μL of the test solution in the Assay as directed under Gas Chromatography according to the following operating conditions. Inject the test solution, increase the column temperature by 20 °C per minute to 140 °C, then increase column temperature by 4 °C per minute to 240 °C, maintain this temperature for not less than 5 minutes, and measure the peak area of chromatogram obtained from the above, calculate the percentage of each related substance by the area

percentag method; each related substance is not more than 0.1 %, total related substance is not more than 0.5 %.

Operating conditions

Detector, column, mobile phase, flow rate and system suitability: Proceed as directed in the operating conditions in the Assay.

(4) ***Monochlorophenols and 2,4-dichlorophenol—*** Weigh accurately 0.25 g of Triclosan, dissolve in 20 mL of acetonitrile, add water to make exactly 25 mL, and use this solution as the test solution. Separately, weigh accurately 5 mg of 4-chlorophenol and 1 mg of 2,4-dichlorophenol, respectively, dissolve in 50 mL of acetonitrile, add water, to make exactly 100 mL. Pipet 1.0 mL of this solution, add a mixture of acetonitrile and water (1 : 1) to make exactly 100 mL, and use this solution as the standard solution. Perfrm the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine the peak areas of each solution by the automatic integration method: the peak areas of 4-chlorophenol and 2,4-dichlorophenol obtained from the test solution are not larger than the peak areas of 4-chlorophenol and 2,4-dichlorophenol obtained from the standard solution.

Operating conditions

Detector : An electrochemical detector (electrode 1: -0.45 V, electrode 2: -0.75 V)

Column : A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for the liquid chromatography (3 to 10 μm in particle size diameter).

Mobile phase : A mixture of acetonitrile and phosphate buffer solution (1 : 1).

Flow rate : 1.0 mL/minute

System suitability

System repeatability : When the test is repeated 5 times with 20 μL each of the standard solution according to the above operating conditions: the relative standard deviation of the peak area obtained from 2,4-dichlorophenol is not more than 9.0 %.

Phosphate buffer solution—Dissolve 1.38 g of anhydrous sodium dihydrogen phosphoric acid and 1.42 g of sodium monohydrogen phosphoric acid, dissolve in water to make 1000 mL.

(5) ***1,3,7-trichlorodibenzo-p-dioxin, 2,8-dichloro dibenzo-p-dioxin, 2,8-dichlorodibenzofuran and 2,4,8-trichlorodibenzofuran—*** Weigh accurately 2.0 g of Triclosan, transfer to a stoppered centrifuge tube, add 5 mL of 2 mol/L potassium hydroxide, and shake for 10 minutes to dissolve. Add 3 mL of *n*-hexane, shake for 10 minutes, and allow the phases to separate. Transfer the organic layer to a suitable container. Add 3 mL of *n*-hexane to the aqueous layer, shake for 10 minutes, and allow the phases to separate. Transfer the organic layer to the previous extract, discard the aqueous layer. Add 3 mL of 2 mol/L potassium hydroxide to the combined organic layers, shake for 10 minutes, and allow the phases to separate. Discard the aqueous layer, add another 3 mL of 2 mol/L potassium hydroxide to the combined organic layers, shake for 10 minutes, and allow the phases to separate. Transfer the organic layer to a suitable container, and evaporate with the aid of a stream of nitrogen to dryness. Dissolve the residue in 1.0 mL of methanol, and use this solution as the test solution. Separately, transfer exactly weighed 5 mg of 2,8-dichlorodibenzofuran and 10 mg of 2,4,8-trichlorodibenzofuran, respectively, to a 100 mL-volumetric flask, add exactly measured 5 mg of 1,3,7-trichlorodibenzo-*p*-dioxin and a quantity equivalent to about 10 mg of 2,8-dichlorodibenzo-*p*-dioxin, and dissolve in methanol to make exactly 100 mL. Pipet 1.0 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine the peak areas of each solution by the automatic integration method: the peak areas of 2,8-dichloro-dibenzofuran, 2,8-dichlorodibenzo-*p*-dioxin, 2,4,8-trichlorodibenzofuran and 1,3,7-trichlorodibenzo-*p*-dioxin obtained from the test solution is not larger than each peak area obtained from the standard solution.

Operating conditions

Detector : An ultraviolet absorption photometer (wavelength : 220 nm).

Column : A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 to 10 μm in partical diameter).

Mobile phase : A mixture of acetonitrile, water and acetic acid (100) (70 : 30 : 0.1).

Flow rate : 1.5 mL/minute

System suitability

System performance : When the procedure is run with 20 μL of standard solution according to the above operating conditions, the relative retention times of peaks obtained from 2,8-dichlorodibenzofuran, 2,8-dichlorodibenzo-*p*-dioxin, 2,4,8-trichlorobenzofuran and 1,3,7-trichlorodibenzo-*p*-dioxin are about 0.59, 0.71, 0.88 and 1.0, respectively.

System repeatability : When the test is repeated 5 times with 20 μL each of the standard solution according to the above operating conditions: the relative standard deviation of peak areas obtained from 2,8-dichloro-dibenzo-*p*-dioxin is not more than 15.0 %.

(6) ***2,3,7,8-tetrachlorodibenzo-p-dioxin and 2,3,7,8-tetrachlorodibenzofuran (extremely toxic)—*** Weigh accurately 30 g of Triclosan, transfer to a separatory funnel, add 30 μL of the internal standard solution, dissolve in 200 mL of 1 mol/L sodium hy-

droxide TS, extract 4 times with 30 mL each of *n*-hexane, and combine all the extracts, Wash the combined extracts with 20 mL of water, extract the washing with 15 mL of *n*-hexane, add the extract to the other combined extracts, add about 3 g of anhydrous sodium sulfate to the combined extracts, allow to stand for 30 minutes, quantitatively transfer to a an appropriate round-bottom flask, and distile, using a distillation apparatus until about 1 mL remains. Transfer this solution to the top of chromatographic column A, and elute with 50 mL of *n*-hexane. Tranfer the eluates to the top of chromatographic column B, and elute with 30 mL of a mixture of *n*-hexane and dichloromethane (98 : 2), discard the elutes. Elute with 40 mL of a mixture of *n*-hexane and dichloromethane (1 : 1), collect the eluates in a round-bottom flask. Distill the combined eluates, using a distillation apparatus until about 1 mL remains. Further concentrate this solution with the aid of a stream of nitrogen to about 50 μL, evaporate at room temperature to dryness, and dissolve in 10 μL of 2,2,4-trimethylpentane, and use this solution as the test solution.

Perform the test with 1 μL of the test solution as directed under Gas Chromatography with high quality Mass Spectrometry, measure the peak areas at mass-to-charge ratios of 319.90, 321.89, 331.88, 333.93, 303.90, 305.90 and 317.94. The peak area for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin at a mass-to-charge ratio of 319.90 is not larger than the peak area of the associated internal standard at a mass-to-charge ratio of 331.88; the peak area for 2,3,7,8-tetrachloro dibenzofuran at a mass-to-charge ratio of 303.90 is not larger than the peak area of the associated internal standard at a mass-to-charge ratio of 315.94.

Operating conditions

Detector: A high-resolution mass spectrograph (electron-impact ionization).

Column: A capillary column, about 0.25 mm in internal diameter and about 60 m in length, coated with cyanopolysiloxane.

Mobile phase: helium

Column temperature: Maintain at 80 °C for 1 minute, raise the temperature to 220 °C at the rate of 20 °C per minute, raise the temperature to 270 °C at the rate of 2 °C per minute, and maintain at 270 °C for 20 minutes.

Signal-to-noise ratio: not less than 50 at a mass-to-charge ratio of 321.89.

Stationary phase A—Take about 10 g of silica gel in a suitable containers, add about 3 mL of 1 mol/L sodium hydroxide TS.

Stationary phase B—Take about 60 g of silica gel in a suitable containers, add about 74 mL of sulfuric acid.

Column A for the chromatography—Transfer 5.1 g of stationary phase A, 0.5 g of silica gel, 6.2 g of stationary phase B, 3.2 g of sodium sulfuric acid in the column, 10 mm in internal diameter, for chromatograph, add 50 mL of *n*-hexane, wash, discard the eluate.

Column B for the chromatography—Transfer 2.5 g of alumina and 2.5 g of sodium sulfate decahydrate in the column, 6 mm in internal diameter, for chromatograph, add 30 mL of *n*-hexane, wash, discard the eluate.

Internal standard solution—Transfer accurately measured quantities of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, ^{13}C-labeled and 2,3,7,8-tetrachlorodibenzofuran, ^{13}C- labeled in nonane, and dilute qustitatively, and stepwise if necessary, with 2,2,4-trimethylpentane to obtain a solution having known concentrations of about 1.0 pg of each per 1 μL .

Water not more than 0.1 % (1 g, volumetric titratioin, direct titration).

Residue of Ignition not more than 0.1 % (1 g).

Assay Weigh accurately about 40 mg each of Triclosan and Triclosan RS, dissolve in dichloromethane to make exactly 10 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with o.5 μL each of test solution and standard solution as directed under Gas Chromatography according to the following operating conditions, calculate the peak areas, A_T and A_S of triclosan obtained from each solution

$$\text{Amount (mg) of triclosan } (C_{12}H_7C_{l3}O_2) = \text{Amount (mg) of Triclosan RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: A hydrogen Flame ionization detector

Column: A tube, about 0.53 mm in internal diameter and about 15 m in length, packed with 50 % of phenyl and 50 % methylpolysiloxan.

Injection port temperature: Maintain at 34 °C and is increased rapidly to 200 °C of the injection.

Column temperature: After the injection, increase the column temperature by 20 °C per minute to 140 °C, then increase column temperature by 4 °C per minute to 240 °C, and maintain at 270 °C for not less than 5 minutes.

Detector temperature: 260 °C

Carrier gas: helium (6 psi)

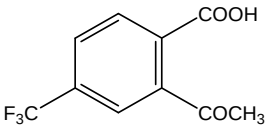
System suitability

System repeatability : When the test is repeated 5 times with 0.5 μL each of standard solution according to the above operating conditions, the relative standard deviation of peak areas obtained from triclosan is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Triflusal



$C_{10}H_7F_3O_4$: 248.16

2-Acetyloxy-4-(trifluoromethyl)benzoic acid [*322-79-2*]

Triflusal contains not less than 98.5 % and not more than 101.5 % of triflusal ($C_{10}H_7F_3O_4$), calculated on the dried basis.

Description Triflusal appears as white powder.
Triflusal is very soluble in ethanol (95), soluble in dichloromethane, and practically insoluble in water.

Melting point—About 118 °C (with decomposition)

Identification (1) Mix about 5 mg of Triflusal and 45 mg of magnesium oxide and ignite them in a crucible until an almost white residue is obtained. Allow to cool, add 1 mL of water, 0.05 mL of phenolphthalein TS and about 1 mL of dilute hydrochloric acid to render the solution colorless. Filter. To a freshly prepared mixture of 0.1 mL of alizarin S TS and 0.1 mL of zirconyl nitrate TS, add 1.0 mL of the filtrate. Mix, allow to stand for 5 minutes and compare the color of the solution with that of a blank prepared in the same manner. The test solution is yellow and the blank is red.

(2) Weigh 0.2 g of Triflusal, add 2.0 mL of dilute sodium hydroxide TS. Heat to boiling and maintain boiling for 15 minutes. Allow to cool and add 25 mL of dilute sulfuric acid. A crystalline precipitate is formed. Filter, wash the precipitate with water and dry at 105 °C. The crystals melt between 176 and 178 °C.

(3) Dissolve 50.0 mg each of Triflusal and Triflusal RS in ethanol (95) to make exactly 100 mL. Add ethanol (95) to 1.0 mL of this solution to make exactly 20 mL. Determine the absorption spectra of these solutions as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared spectra of Triflusal and Triflusal RS as directed in potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity (1) ***Clarity of solution***— Dissolve about 1.0 g of Triflusal in ethanol (95) to make 20 mL: the solution is clear.

(2) ***Heavy metals***— Weigh 2.0 g of Triflusal, dissolve in 9 mL of ethanol (95), add water to make 20 mL, add 2 mL of dilute acetic acid and a mixture of ethanol (95) and water (9 : 6) to make 50 mL, and perform the test using this solution as the test solution. Prepare the cotrol solution as follows: add 2 mL of dilute acetic acid and a mixture of ethanol (95) and water (9 : 6) to 2.0 mL of lead standard solution to make 50 mL (not more than 10 ppm).

(3) ***Triflusal related substance I***—Weigh exactly 0.1 g of Triflusal, dissolve in the mobile phase to make exactly 25 mL, and use this solution as the test solution. Separately, weigh exactly 40 mg of Triflusal related substance I (2-acetoxyterephthalic acid) RS, dissolve in the mobile phase to make exactly 100 mL, add the mobile phase to 1.0 mL of this solution to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following operating conditions. The peak area of 2-acetoxyterephthalic acid obtained from the test solution is not greater than the area of principal peak obtained from the standard solution (not more than 0.1 %).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 250 nm)

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with aminopropylsilanized silica gel for the liquid chromatography (5 μm in particle diameter).

Mobile phase: A mixture of acetonitrile and 0.05 mol/L phosphate buffer solution (pH 4.5) (75 : 25).

Flow rate : 1.2 mL/minute

System suitability

When the procedure is run with 20 μL of the mixture of the same volumes of the test solution and the standard solution, according to the above operating conditions, the retention times of triflusal and 2-acetoxy-terephthalic acid are about 2.4 and 5 minutes, respectively.

(4) ***Triflusal related substance II***—Dissolve 0.1 g of Triflusal in 15 mL of ethanol (95). Add 15 mL of cold water and 0.5 mL of 5 w/v % ammonium iron (III) sulfate solution. Allow to stand for 1 minute. This solution is not more intensely colored than that of a control solution as the follows: dissolve 10.0 mg of Triflusal related substance (II) [4-(trifluoromethylsalicylic acid)] in ethanol (95) to make exactly 100 mL. To 3 mL of this solution, add 0.1 mL of acetic acid (100), 0.5 mL of 0.5 w/v % ammonium iron (III) sulfate solution, 12 mL of ethanol (95) and 15 mL of water.

Loss on Drying Not more than 0.5 % (1 g, in vacuum, P_2O_5).

Residue on Ignition Not more than 0.1 % (1 g, platinum crucible).

Assay Weigh accurately about 0.2 g of Triflusal, dissolve in 50 mL of ethanol (95), and titrate with 0.1

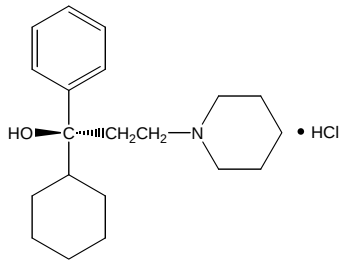
mol/L sodium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

1 mL of 0.1 mol/L sodium hydroxide VS
= 24.82 mg of $C_{10}H_7F_3O_4$

Containers and Storage ***Containers***—Tight containers.

Storage—Not exceeding 25 °C.

Trihexyphenidyl Hydrochloride



and enantiomer

$C_{20}H_{31}NO \cdot HCl$: 337.93

1-Cyclohexyl-1-phenyl-3-piperidin-1-ylpropan-1-ol hydrochloride [*52-49-3*]

Trihexyphenidyl Hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO \cdot HCl$).

Description Trihexyphenidyl Hydrochloride is a white crystalline powder, is odorless and has a bitter taste.

Trihexyphenidyl Hydrochloride is soluble in ethanol (95), sparingly soluble in acetic acid (100), slightly soluble in water, very slightly soluble in acetic anhydride, and practically insoluble in ether.

Melting point—About 250 °C (with decomposition).

Identification (1) Dissolve 1 g of Trihexyphenidyl Hydrochloride in 100 mL of water by warming, cool and use this solution as the test solution. To 5 mL of the test solution, add 1 mL of a solution of 2,4,6-trinitrophenol in chloroform (1 in 50) and shake vigorously: a yellow precipitate is produced.

(2) To 20 mL of the test solution obtained in (1), add 2 mL of sodium hydroxide TS: a white precipitate is produced. Collect the precipitate, wash with a small volume of water, recrystallize from methanol and dry in a desiccator (in vacuum, silica gel) for 2 hours: the crystals so obtained melt between 113 °C and 117 °C.

(3) The test solution obtained in (1) responds to the Qualitative Tests (2) for chloride.

pH Dissolve 1.0 g of Trihexyphenidyl Hydrochloride in 100 mL of water by warming and cool: the pH of this solution is between 5.0 and 6.0.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Trihexyphenidyl Hydrochloride in 100 mL of water by warming: the solution is clear and colorless.

(2) ***Heavy metals***—Dissolve 1.5 g of Trihexyphenidyl Hydrochloride in 60 mL of water by warming in a water-bath at 80 °C, cool and filter. To 40 mL of the filtrate, add 2 mL of dilute acetic acid and water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of standard lead solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(3) ***Related substances***—Weigh accurately 20 mg of Trihexyphenidyl Hydrochloride, dissolve in 10 mL of the mobile phase, and use this solution as the test solution. To 1.0 mL of the test solution add the mobile phase to make 200 mL, pipet 10 mL of this solution, add the mobile phase to make 50 mL, and use this solution as the standard solution (1). Separately, weigh accurately about 10 mg of Trihexyphenidyl Related Substance I {1-phenyl-3-(pyperidin-1-yl)propan-1-one} RS, dissolve in 10 mL of the mobile phase, and use this solution as the standard solution (2). Pipet 1.0 mL of the standard solution (2), add the mobile phase to make 100 mL, and use this solution as the standard solution (3). Pipet 1 mL of the standard solution (2) and 1 mL of the test solution, add the mobile phase to make 100 mL, and use this solution as the standard solution (4). Perform the test with 20 μL each of the test solution and standard solutions (1), (3), and (4) as directed under Liquid Chromatography according to the following conditions. The peak area of trihexyphenidyl related substance I obtained from the test solution is not larger than the area of the principal peak from the standard solution (3) (not more than 0.5 %), the area of any other related substance is not larger than the area of the principal peak from the standard solution (1) (not more than 0.1 %), and the total area of related substances is not more than 0.5 %. Exclude any peak with an area less than 0.2 times the area of the principal peak from the standard solution (1) (0.02 %).

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm)

Column: A stainless steel column about 5 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Temperature: A constant temperature of about 45 °C

Mobile phase: Mix 200 mL of water with 0.2 mL of triethylamine, and adjust the pH to 4.0 with phosphoric acid. To this solution add 800 mL of acetonitrile.

Flow rate: 1.0 mL/minute

System suitability

System performance: When the procedure is run with 20 μL of the standard solution (4) under the above

operating conditions, the resolution between the peaks of trihexyphenidyl and trihexyphenidyl related substance I is not less than 4.0.

Time span of measurement: About 3 times as long as the retention time of trihexyphenidyl.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.5 g of Trihexyphenidyl Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (1 : 1) and titrate with 0.1 mol/L perchloric acid-dioxane VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid-dioxane VS
= 33.793 mg of $C_{20}H_{31}NO \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Trihexyphenidyl Hydrochloride Tablets

Trihexyphenidyl Hydrochloride Tablets contain not less than 93.0 % and not more than 107.0 % of the labeled amount of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO \cdot HCl$: 337.93).

Method of Preparation Prepare as directed under Tablets, with Trihexyphenidyl Hydrochloride.

Identification (1) Weigh a portion of powdered Trihexyphenidyl Hydrochloride Tablets, equivalent to 0.1 g of trihexyphenidyl hydrochloride according to the labeled amount, add 30 mL of chloroform, shake and filter. Evaporate the filtrate on a water-bath to dryness. Dissolve the residue in 10 mL of water by warming, cool and use this solution as the test solution. With 5 mL of the test solution, proceed as directed in the Identification (1) under Trihexyphenidyl Hydrochloride.

(2) Weigh a portion of powdered Trihexyphenidyl Hydrochloride Tablets, equivalent to 10 mg of trihexyphenidyl hydrochloride according to the labeled amount, add 5 mL of chloroform, shake, filter and use the filtrate as the test solution. Separately, dissolve 20 mg of trihexyphenidyl hydrochloride RS in 10 mL of chloroform and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (9 : 1) to a distance of about 10 cm and air-dry the plate. Spray evenly hexachloroplatinic (IV) acid-potassium iodide TS on the plate: the spots from the test solution and standard solution show a blue-purple color and the same R_f value.

(3) The test solution obtained in (1) responds to the Qualitative Tests (2) for chloride.

Dissolution Test Perform the test with 1 tablet of Trihexyphenidyl Hydrochloride Tablets at 50 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of diluted phosphate buffer solution, pH 6.8 (1 in 2) as the dissolution solution. Take 30 mL or more of the dissolved solution after 30 minutes from the start of the test and filter through a membrane filter with a pore size of not more than 0.8 μm. Discard the first 10 mL of the filtrate and use the subsequent filtrate as the test solution. Separately, weigh accurately about 10 mg of Trihexyphenidyl Hydrochloride RS, previously dried at 105 °C for 3 hours and dissolve in diluted phosphate buffer solution, pH 6.8 (1 in 2) to make exactly 100 mL. Pipet 2 mL of this solution, add diluted phosphate buffer solution, pH 6.8 (1 in 2) to make exactly 100 mL and use this solution as the standard solution. Pipet 20 mL each of the test solution, the standard solution and diluted phosphate buffer solution, pH 6.8 (1 in 2), add exactly 1 mL of diluted acetic acid (1 in 10), immediately add 5.0 mL of bromocresol green-sodium hydroxide-acetic acid-sodium acetate TS and shake. Then, add exactly 10 mL each of dichloromethane, shake well, centrifuge and take the dichloromethane layer. Determine the absorbances, A_T, A_S and A_B, for the test solution, the standard solution, and the diluted phosphate buffer solution pH 6.8 (1 in 2), respectively, of these dichloromethane layers at 415 nm as directed under Ultraviolet-visible Spectrophotometry, using dichloromethane as a blank.
The dissolution rate of Trihexyphenidyl Hydrochloride Tablets in 30 minutes should be not less than 70 %.

Dissolution rate (%) with respect to the labeled amount of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO \cdot HCl$)

$$= W_S \times \frac{A_T - A_B}{A_S - A_B} \times \frac{1}{C} \times 18$$

W_S: Amount (mg) of Trihexyphenidyl Hydrochloride RS.

C: Labeled amount (mg) of Trihexyphenidyl Hydrochloride in 1 tablet.

Uniformity of Dosage Units It meets the requirement when the content uniformity test is performed according to the following procedure.
To one tablet of Trihexyphenidyl Hydrochloride Tablets, add 2 mL of dilute hydrochloric acid and 60 mL of water, disintegrate by vigorous shaking for 10 minutes and warm in a water-bath with occasional shaking for 10 minutes. Cool, add 2 mL of methanol and add water to make exactly V mL of the solution containing about

20 g of trihexyphenidyl hydrochloride per mL. Centrifuge, if necessary and use the clear supernatant liquid as the test solution. Separately, dissolve about 20 mg of trihexyphenidyl hydrochloride RS (previously determine the loss on drying at 105 °C for 3 hours) in methanol to make exactly 20 mL. Pipet 2 mL of this solution, add 2 mL of dilute hydrochloric acid and water to make exactly 100 mL and use this solution as the standard solution. Pipet 10 mL each of the test solution and the standard solution, transfer to glass-stoppered centrifuge tubes, add exactly 10 mL of bromocresol purple-dipotassium hydrogen phosphate-citric acid TS and 15 mL of chloroform, stopper tightly, shake well and centrifuge. Pipet 10 mL each of the chloroform layers, add chloroform to make exactly 50 mL. Determine the absorbances, A_T and A_S, of these solutions obtained from the test solution and the standard solution at 408 nm as directed under Ultraviolet-visible Spectrophotometry, respectively.

$$\text{Amount (mg) of trihexyphenidyl hydrochloride } (C_{20}H_{31}NO\cdot HCl) = \text{Amount (mg) of Trihexyphenidyl Hydrochloride RS, calculated on the dried basis} \times \frac{A_T}{A_S} \times \frac{V}{1000}$$

Assay Add 20 Trihexyphenidyl Hydrochloride Tablets to an amount of 0.1 mol/L hydrochloric acid, equivalent to 10 % of the total volume, sonicate for 10 minutes to disintegrate completely, add an amount of the mobile phase, equivalent to 40 % of the total volume, shake for 10 minutes, cool at an ordinary temperature, add 50 % of the total volume, mix, filter so that each mL contains 0.2 mg of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO\cdot HCl$) according to the labeled amount, and use as the test solution. Separately, weigh accurately about 20 mg of Trihexyphenidyl Hydrochloride RS, dissolve in the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of trihexyphenidyl hydrochloride in each solution.

$$\text{Amount (mg) of trihexyphenidyl hydrochloride } (C_{20}H_{31}NO\cdot HCl) = \text{Amount (mg) of Trihexyphenidyl Hydrochloride RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 8 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Mobile phase: Adjust the pH of a mixture of acetonitrile, water, and triethylamine (920 : 80 : 0.2) to 4.0 with phosphoric acid.

Flow rate: 0.2 mL/minute

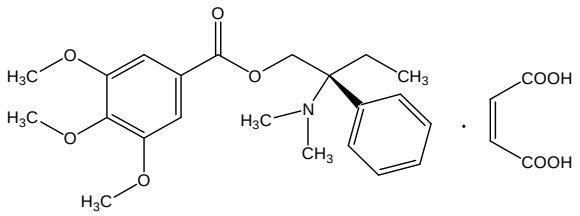
System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of trihexyphenidyl hydrochloride are not less than 1300 and not more than 3.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of isoniazid is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Trimebutine Maleate



and enantiomer

$C_{22}H_{29}NO_5\cdot C_4H_4O_4$: 503.54

(*Z*)-but-2-Enedioic acid; [2-(Dimethylamino)-2-phenylbutyl]3,4,5-trimethoxybenzoate [*34140-59-5*]

Trimebutine Maleate, when dried, contains not less than 98.5 % and not more than 101.0 % of trimebutine maleate ($C_{22}H_{29}NO_5\cdot C_4H_4O_4$).

Description Trimebutine Maleate appears as white crystals or powder.

Trimebutine Maleate is freely soluble in *N,N*-dimethylformamide or in acetic acid (100), soluble in acetonitrile, and slightly soluble in water or in ethanol (99.5).

Trimebutine Maleate dissolves in 0.01 mol/L hydrochloric acid TS.

A solution of Trimebutine Maleate (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectra of solutions of Trimebutine Maleate and Trimebutine Maleate RS in 0.01 mol/L hydrochloric acid TS (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Trimebutine Maleate and Trimebutine Maleate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting Point 131 ~ 135 °C.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Trimebutine Maleate according to Method 2 under Heavy Metals Limit Test, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Arsenic***—Prepare the test solution with 2.0 g of Trimebutine Maleate according to Method 3, and perform the test (not more than 1 ppm).

(3) ***Related substances***—Dissolve 0.10 g of Trimebutine Maleate in 100 mL of a mixture of 0.01 mol/L hydrochloric acid TS and acetonitrile (13 : 7), and use this solution as the test solution. Pipet 1.0 mL of the test solution, add a mixture of 0.01 mol/L hydrochloric acid TS and acetonitrile (13 : 7) to make exactly 250 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas of each solution by the automatic integration method: the area of each peak other than maleic acid and trimebutine from the test solution is not larger than 0.5 times the peak area of trimebutine from the standard solution, and the total area of the peaks other than maleic acid and trimebutine from the test solution is not larger than the peak area of trimebutine from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: Dissolve 1 g of sodium 1-pentanesulfonate in 650 mL of diluted perchloric acid (17 in 20000), previously adjusted to pH 3.0 with a solution of ammonium acetate (1 in 1000). To 650 mL of this solution add 350 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of trimebutine is about 9 minutes.

System suitability

Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of 0.01 mol/L hydrochloric acid TS and acetonitrile (13 : 7) to make exactly 20 mL. Confirm that the peak area of trimebutine obtained from 20 μL of this solution is equivalent to 20 to 30 % of that from the standard solution.

System performance: Dissolve 40 mg of Trimebutine Maleate and 20 mg of imipramine hydrochloride in 100 mL of a mixture of 0.01 mol/L hydrochloric acid and acetonitrile (13 : 7). When the procedure is run with 20 μL of this solution under the above operating conditions, trimebutine and imipramine are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trimebutine is not more than 5.0 %.

Time span of measurement: About 2 times as long as the retention time of trimebutine, beginning after the peak of maleic acid.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 3 hours).

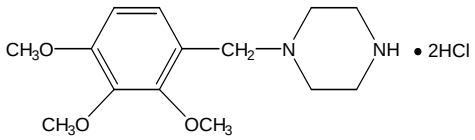
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.8 g of Trimebutine Maleate, previously dried, dissolve in 70 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (indicator: 3 drops of crystal violet TS) until the color of the solution changes from purple through blue to blue-green. Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 50.35 mg of $C_{22}H_{29}NO_5 \cdot C_4H_4O_4$

Containers and Storage ***Containers***—Well-closed containers.

Trimetazidine Hydrochloride



$C_{14}H_{22}N_2O_3 \cdot 2HCl$: 339.26

1-[(2,3,4-Trimethoxyphenyl)methyl]piperazine dihydrochloride [*13171-25-0*]

Trimetazidine Hydrochloride contains not less than 98.0 % and not more than 101.0 % of trimetazidine hydrochloride ($C_{14}H_{22}N_2O_3 \cdot 2HCl$), calculated on the anhydrous basis.

Description Trimetazidine Hydrochloride appears as white crystalline powder.

Trimetazidine Hydrochloride is very soluble in water or in formic acid, soluble in methanol and practically insoluble in ether.

pH—Dissolve 1.0 g of Trimetazidine Hydrochloride in 20 mL of water: the pH of this solution is between 2.3 and 3.3.

Melting point—About 227 °C (with decomposition).

Identification (1) Determine the absorption spectra of solutions of Trimetazidine Hydrochloride and Trimetazidine Hydrochloride RS in 0.1 mol/L hydrochloric acid TS (1 in 6250) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Trimetazidine Hydrochloride and Trimetazidine Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Trimetazidine Hydrochloride (1 in 50) responds to the Qualitative Tests for chloride.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Trimetazidine Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Dissolve 0.2 g of Trimetazidine Hydrochloride in 50 mL of water, and use this solution as the test solution. Pipet 2 mL of the test solution, add water to make exactly 20 mL, pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas of the test solution and standard solution by the automatic integration method: the area of any peak other than trimetazidine from the test solution is not larger than 1.5 times the peak area of trimetazidine from the standard solution, and the total area of the peaks other than trimetazidine from the test solution is not larger than 2.5 times the peak area of trimetazidine from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 240 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: A mixture of a solution prepared by dissolving 2.87 g of sodium 1-heptanesulfonate in water to make 1000 mL and adjusting the pH to 3.0 with diluted phosphoric acid (1 in 10), and methanol (3 : 2)

Mobile phase B: Methanol

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-50	95→75	5→25

Flow rate: Adjust the flow rate so that the retention time of trimetazidine is about 25 minutes.

Time span of measurement: About 2 times as long as the retention time of trimetazidine, beginning after the solvent peak.

System suitability

Test for required detectability: Pipet 5 mL of the standard solution, and add water to make exactly 20 mL. Confirm that the peak area of trimetazidine obtained from 10 μL of this solution is equivalent to 18 to 32 % of that from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of trimetazidine are not less than 15000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trimetazidine is not more than 2.0 %.

Water Not more than 1.5 % (2 g, volumetric titration, direct titration).

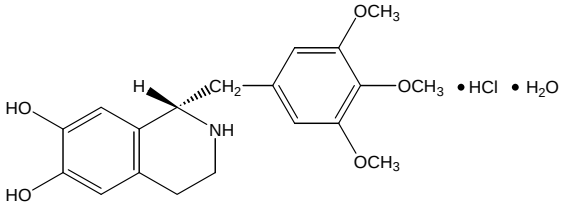
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.12 g of Trimetazidine Hydrochloride, dissolve in 5 rnL of formic acid, add exactly 15 mL of 0.1 mol/L perchioric acid VS, and heat at 90 °C to 100 °C for 30 minutes. After cooling, add 45 mL of acetic acid (100), and titrate the excess perchioric acid with 0.1 mol/L sodium acetate VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 16.963 mg of $C_{14}H_{22}N_2O_3 \cdot 2HCl$

Containers and Storage ***Containers***—Tight containers.

Trimetoquinol Hydrochloride Hydrate



Tretoquinol Hydrochloride

$C_{19}H_{23}NO_5 \cdot HCl \cdot H_2O$: 399.87

1-(3,4,5-Trimethoxybenzyl)-1,2,3,4-tetrahydro-6,7-isoquinolinediol hydrate hydrochloride
[*8559-59-6,* anhydride]

Trimetoquinol Hydrochloride Hydrate contains not less than 98.0 % and not more than 101.0 % of trimetoquinol hydrochloride ($C_{19}H_{23}NO_5 \cdot HCl$: 381.85), calculated on the anhydrous basis.

Description Trimetoquinol Hydrochloride appears as white crystals or crystalline powder and is odorless.
Trimetoquinol Hydrochloride Hydrate is freely soluable in methanol, and sparingly soluble in water or in ethanol (99.5).

Melting point—About 151 °C (with decomposition, after drying, in vacuum, at 105 °C, for 4 hours).

Identification (1) Determine the absorption spectra of solutions of Trimetoquinol Hydrochloride Hydrate and Trimetoquinol Hydrochloride Hydrate RS in 0.01 mol/L hydrochloric acid TS (1 in 20000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared spectra with Trimetoquinol Hydrochloride Hydrate and Trimetoquinol Hydrochloride Hydrate RS as directed in the potassium chloride disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(5) A solution of Trimetoquinol Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests (1) for chloride.

Specific Optical Rotation $[\alpha]_D^{20}$: -16 ~ -19° (calculated on the dried basis, 0.25 g, water, warming and cooling, 25 mL, 100 mm).

pH Dissolve 1.0 g of Trimetoquinol Hydrochloride Hydrate in 100 mL of water by warming and cool: the pH of this solution is between 4.5 and 5.5.

Purity (1) ***Clarity and color of solution***—Dissolve 0.10 g of Trimetoquinol Hydrochloride Hydrate in 10 mL of water by warming: the solution is clear and colorless.

(2) ***Sulfate***—Perform the test with 0.5 g of Trimetoquinol Hydrochloride Hydrate. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038 %).

(3) ***Heavy metals***—Proceed with 1.0 g of Trimetoquinol Hydrochloride Hydrate according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(4) ***Related substances***—Dissolve 50 mg of Trimetoquinol Hydrochloride Hydrate in 50 mL of the mobile phase and use this solution as the test solution. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and the standard solution, as directed under Liquid Chromatography according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than that of trimetoquinol from the test solution is not larger than the peak area of trimetoquinol from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 283 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: Dissolve 2 g of potassium dihydrogen phosphate and 2 g of sodium 1-pentane sulfonate in 1000 mL of water. Adjust with phosphoric acid to a pH between 2.8 and 3.2, filter through a membrane filter (0.4 μm in pore size) and add 200 mL of acetonitrile to 800 mL of the filtrate.

Flow rate: Adjust the flow rate so that the retention time of trimetoquinol is about 7 minutes.

System suitability

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of trimetoquinol obtained from 20 μL of this solution is equivalent to 7 to 13 % of that of trimetoquinol obtained from 20 μL of the standard solution.

System performance: Dissolve 5 mg of Trimetoquinol Hydrochloride Hydrate and 1 mg of procaine hydrochloride in 50 mL of the mobile phase. When the procedure is run with 20 μL of this solution under the above operating conditions, procaine and trimetoquinol are eluted in this order with the resolution between their peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trimetoquinol is not more than 2.0 %.

Time span of measurement: About twice as long as the retention time of trimetoquinol after the solvent peak.

Water 3.5 ~ 5.5 % (0.3 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.5 g each of Trimetoquinol Hydrochloride Hydrate, dissolve in 2 mL of 0.1 mol/L hydrochloric acid VS and 70 mL of ethanol (99.5) with thorough shaking and titrate with 0.1 mol/L potassium hydroxide-ethanol VS (potenti-

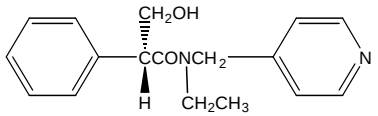
ometric titration, Endpoint Detection Method in Titrimetry). Calculate the consumed volume of 0.1 mol/L potassium hydroxide-ethanol VS between the first inflection point and the second inflection point.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 38.185 mg of $C_{19}H_{23}NO_5 \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Tropicamide



and enantiomer

$C_{17}H_{20}N_2O_2$: 284.35

N-Ethyl-3-hydroxy-2-phenyl-*N*-(pyridin-4-ylmethyl)propanamide [*1508-75-4*]

Tropicamide, when dried, contains not less than 98.5 % and not more than 101.0 % of tropicamide ($C_{17}H_{20}N_2O_2$).

Description Tropicamide is a white, crystalline powder, is odorless and has a bitter taste.
Tropicamide is freely soluble in ethanol (95) or in chloroform, slightly soluble in water or in ether, and practically insoluble in petroleum ether.
Tropicamide dissolves in dilute hydrochloric acid.

pH—The pH of a solution of Tropicamide (1 in 500) is between 6.5 and 8.0.

Identification (1) To 5 mg of Tropicamide, add 0.5 mL of a solution of ammonium vanadate in sulfuric acid (1 in 200) and heat: a blue-purple color is observed.

(2) Dissolve 5 mg of Tropicamide in 1 mL of ethanol (95) and 1 mL of water, add 0.1 g of 1-chloro-2,4-dinitrochlorobenzene and heat in a water-bath for 5 minutes. Cool and add 2 to 3 drops of a solution of sodium hydroxide (1 in 10) and 3 mL of ethanol (95): a red-purple color is observed.

Melting Point 96 ~ 99 °C.

Absorbance $E_{1cm}^{1\%}$ (255 nm): 166 ~ 180 (after drying, 5 mg, 2 mol/L hydrochloric acid TS, 200 mL).

Purify (1) ***Chloride***—Dissolve 1.0 g of Tropicamide in 30 mL of ethanol (95), add 6 mL of dilute nitric acid and water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution with 0.45 mL of 0.01 mol/L hydrochloric acid VS, 30 mL of ethanol (95), 6 mL of dilute nitric acid and add water to make 50 mL (not more than 0.016 %).

(2) ***Heavy metals***—Dissolve 1.0 g of Tropicamide in 30 mL of ethanol (95), add 2 mL of dilute acetic acid and water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of standard lead solution, 30 mL of ethanol (95), 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(3) ***N-Ethyl-γ-picolylamine***—Dissolve 0.10 g of Tropicamide in 5 mL of water by heating, add 1 mL of a solution of acetaldehyde (1 in 20) and shake well. Add 1 to 2 drops of sodium pentacyanonitrosylferrate (III) TS and 1 to 2 drops of sodium bicarbonate TS and shake: no blue color is observed.

(4) ***Tropic acid***—To 10 mg of Tropicamide, add 5 mg of sodium borate and 7 drops of 4-dimethylaminobenzaldehyde TS and heat on a water-bath for 3 minutes. Cool in ice-water and add 5 mL of acetic anhydride: no red-purple color is observed.

Loss on Drying Not more than 0.3 % (1 g, in vacuum, silica gel, 24 hours).

Residue on Ignition Not more than 0.1 % (1 g).

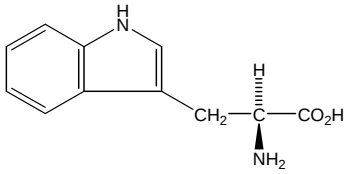
Assay Weigh accurately about 0.5 g of Tropicamide, previously dried, dissolve in 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (indicator: 3 drops of methylrosaniline chloride TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 28.435 mg of $C_{17}H_{20}N_2O_2$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

L-Tryptophan



$C_{11}H_{12}N_2O_2$: 204.23

(2*S*)-2-Amino-3-(1*H*-indol-3-yl)propanoic acid [*73-22-3*]

L-Tryptophan, when dried, contains not less than 98.5 % and not more than 101.0 % of L-tryptophan ($C_{11}H_{12}N_2O_2$).

Description L-Tryptophan appears as white to yellowish white crystals or crystalline powder, is odorless and has a slightly bitter taste.
L-Tryptophan is freely soluble in formic acid, slightly soluble in water, very slightly soluble in ethanol (95) and practically insoluble in ether.
L-Tryptophan dissolves in dilute hydrochloric acid.

Identification Determine the infrared spectra of L-Tryptophan and L-Tryptophan RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Optical Rotation $[\alpha]_D^{20}$: -30.0 ~ -33.0°. Weigh accurately about 0.25 g of L-Tryptophan, previously dried and dissolve in 20 mL of water by warming. After cooling, add water to make exactly 25 mL and determine the specific rotation of the solution in a 100 mm cell.

pH Dissolve 1.0 g of L-Tryptophan in 100 mL of water by warming and cool: the pH of this solution is between 5.4 and 6.4.

Purity (1) ***Clarity of solution***— Dissolve 0.20 g of L-Tryptophan in 10 mL of 2 mol/L hydrochloric acid TS: the solution is clear.

(2) ***Chloride***—Dissolve 0.5 g of L-Tryptophan in 6 mL of dilute nitric acid and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021 %).

(3) ***Sulfate***— Dissolve 0.6 g of L-Tryptophan in 40 mL of water and 1 mL of dilute hydrochloric acid and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028 %).

(4) ***Ammonium***—Perform the test with 0.25 g of L-Tryptophan. Prepare the control solution with 5.0 mL of standard ammonium solution (not more than 0.02 %).

(5) ***Heavy metals***—Proceed with 1.0 g of L-Tryptophan according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(6) ***Iron***—Dissolve 0.333 g of L-Tryptophan in water to make 45 mL, add 2 mL of hydrochloric acid, and use this solution as the test solution. To 1.0 mL of standard iron solution add water to make 45 mL, add 2 mL of hydrochloric acid, and use this solution as the standard solution. To the test solution and standard solution add 50 mg of ammonium peroxydisulfate and 3 mL of 30 % ammonium thiocyanide solution, and mix: the color obtained from the test solution is not more intense than that from the standard solution (mot more than 30 ppm).

(7) ***Arsenic***—Dissolve 1.0 g of L-Tryptophan in 3 mL of 1 mol/L hydrochloric acid TS and 2 mL of water by heating and perform the test (not more than 2 ppm).

(8) ***Related substances***— Dissolve 0.30 g of L-Tryptophan in 1 mL of 1 mol/L hydrochloric acid TS, add water to make 50 mL and use this solution as the test solution. Pipet 1 mL of the test solution and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography, Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3 : 1 : 1) to a distance of about 10 cm and air-dry the plate at 80 °C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate and heat at 80 °C for 5 minutes: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.3 % (1 g, 105 °C, 3 hours).

Residue on Igntion Not more than 0.1 % (1 g).

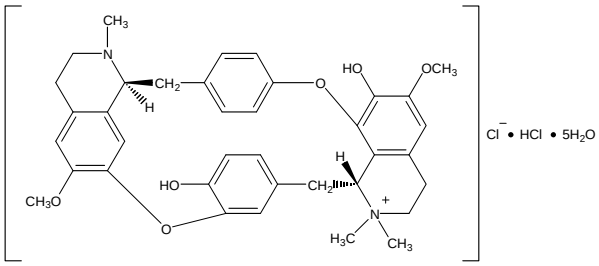
Assay Weigh accurately about 0.2 g of L-Tryptophan, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 20.423 mg of $C_{11}H_{12}N_2O_2$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Tubocurarine Chloride Hydrochloride Hydrate



Tubocurarine Hydrochloride
$C_{37}H_{41}ClN_2O_6 \cdot HCl \cdot 5H_2O$: 771.72

(1*S*,16*R*)-9,21-Dihydroxy-10,25-dimethoxy-15,15,30-trimethyl-7,23-dioxa-15,30-diazaheptacyclo [22.6.2.2^{3,6}.1^{8,12}.1^{18,22}.0^{27,31}.0^{16,34}]hexatriaconta-3,5,8,10,12(34),18(33),19,21,24(32),25,27(31),35-dodecaen-15-ium chloride, hydrochloride, pentahydrate [*6989-98-6*]

Tubocurarine Chloride Hydrochloride Hydrate contains not less than 98.0 % and not more than 101.0 % of tubocurarine chloride hydrochloride ($C_{37}H_{41}ClN_2O_6 \cdot HCl$: 681.65), calculated on the dried basis.

Description Tubocurarine Chloride Hydrochloride Hydrate appears as white crystals or crystalline powder and is odorless.

Tubocurarine Chloride Hydrochloride Hydrate is sparingly soluble in water or in ethanol (95), slightly soluble in acetic acid (100), and practically insoluble in ether or in chloroform.

pH—The pH of a solution of Tubocurarine Chloride Hydrochloride Hydrate (1 in 100) is between 4.0 and 6.0.

Melting point—About 270 °C (with decomposition).

Identification (1) To 20 mL of a solution of Tubocurarine Chloride Hydrochloride Hydrate (1 in 2000), add 0.2 mL of sulfuric acid and 2 mL of a solution of potassium iodate (1 in 100), shake and heat in a water-bath for 30 minutes: a yellow color is observed.

(2) To 1 mL of a solution of Tubocurarine Chloride Hydrochloride Hydrate (1 in 100), add 1 mL of a solution of Reinecke salt monohydrate (1 in 100): a red precipitate is produced.

(3) Determine the absorption spectra of solutions of Tubocurarine Chloride Hydrochloride Hydrate and Tubocurarine Chloride Hydrochloride Hydrate RS (3 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) A solution of Tubocurarine Chloride Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests (2) for chloride.

Specific Optical Rotation $[\alpha]_D^{20}$: +210 ~ +220° (calculated on the dried basis, 0.1 g, , water, 10 mL, after allowing to stand for 3 hours, 100 mm).

Purity (1) ***Clarity and color of solution***—Dissolve 0.1 g of Tubocurarine Chloride Hydrochloride Hydrate in 10 mL of ethanol (95): the solution is clear and colorless.

(2) ***Chloroform-soluble substances***—Weigh accurately about 0.2 g of Tubocurarine Chloride Hydrochloride Hydrate, calculated on the dried basis, add 200 mL of water and 1 mL of a saturated solution of sodium bicarbonate and extract with three 20 mL volumes of chloroform. Combine the chloroform extracts, wash with 10 mL of water, filter the chloroform solution through absorbent cotton into a tared beaker, wash the absorbent cotton with two 5 mL volumes of chloroform and combine the filtrate and the washings. Evaporate the chloroform in a water-bath and dry the residue at 105 °C for 1 hour: the mass of the residue is not more than 2.0 % of the mass of Tubocurarine Chloride Hydrochloride Hydrate taken. Add 10 mL of water to the residue: the residue does not dissolve. Add 1 mL of hydrochloric acid and stir: the residue dissolves.

(3) ***Related substances***—Weigh accurately 30 mg of Tubocurarine Chloride Hydrochloride Hydrate, add the mobile phase to make exactly 100 mL, and use this solution as the test solution. Perform the test with 10 μL of the test solution as directed in the area percentage method under Liquid Chromatography according to the following conditions, and determine each peak area other than the principal peak obtained from the test solution, A_i, and the total area of all peaks, A_S: the total amount of related substances is not more than 5.0 %.

$$\text{Amount (\%) of each related substance} = 100 \times \frac{A_i}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (1.5 to 10 μm in particle diameter).

Mobile phase: Mix 270 mL of a mixture of acetonitrile and methanol (3 : 2) with 20 mL of 25 % tetramethylammonium hydroxide-methanol TS, add water to make 1000 mL, and adjust the pH to 4.0 with phosphoric acid.

Flow rate: 1 mL/minute

System suitability

System performance: Weigh accurately 30 mg of Tubocurarine Chloride Hydrochloride RS and 50 mg of phenol, dissolve in 100 mL of the mobile phase so that each mL contains 0.3 mg and 0.5 mg, respectively, and use this solution as the system suitability solution. When the procedure is run with 10 μL of this solution under the above operating conditions, the relative retention time of tubocurarine hydrochloride with respect to phenol is about 0.50, the resolution between these peaks is not less than 2.0, and the symmetry factor of the peak of tubocurarine chloride is not more than 2.0.

System repeatability: Weigh accurately 30 mg of Tubocurarine Chloride Hydrochloride RS, and dissolve in 100 mL of the mobile phase so that each mL contains 0.3 mg. When the test is repeated 5 times with 10 μL each of this solution under the above operating conditions, the relative standard deviation of the peak area of tubocurarine chloride is not more than 2.0 %.

Loss on Drying 9 ~ 12 % (0.5 g, in vacuum, P_2O_5,

105 °C, 4 hours).

Residue on Ignition Not more than 0.2 % (0.5 g).

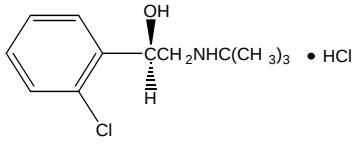
Assay Weigh accurately about 0.5 g of Tubocurarine Chloride Hydrochloride Hydrate, add 20 mL of acetic acid (100) and dissolve by warming in a water-bath. After cooling, add 60 mL of acetic anhydride and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 34.082 mg of $C_{37}H_{41}ClN_2O_6 \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Tulobuterol Hydrochloride



and enantiomer

$C_{12}H_{18}ClNO \cdot HCl$: 264.19

1-(2-Chlorophenyl)-2-[(2-methyl-2-propanyl)amino]ethanol hydrochloride [*56776-01-3*]

Tulobuterol Hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of tulobuterol hydrochloride ($C_{12}H_{18}ClNO \cdot HCl$).

Description Tulobuterol Hydrochloride appears as white crystals or crystalline powder.
Tulobuterol Hydrochloride is very soluble in methanol, freely soluble in water, in ethanol (95) or in acetic acid (100), sparingly soluble in acetic anhydride, and very slightly soluble in ether.
A solution of Tulobuterol Hydrochloride (1 in 20) shows no optical rotation.

Melting point—About 163 °C.

Identification (1) Determine the absorption spectra of solutions of Tulobuterol Hydrochloride and Tulobuterol Hydrochloride RS (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Tulobuterol Hydrochloride and Tulobuterol Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution of Tulobuterol Hydrochloride (1 in 20) responds to the Qualitative Tests for chloride.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Tulobuterol Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Proceed with 2.0 g of Tulobuterol Hydrochloride according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Dissolve 0.30 g of Tulobuterol Hydrochloride in 5 mL of methanol and use this solution as the test solution. Pipet 1 mL of the test solution, add methanol to make exactly 50 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Use a plate of silica gel with a fluorescent indicator for thin-layer chromatography previously developed with the upper-layer of a mixture of ethyl acetate and ammonia solution (28) (200 : 9) to the top of the plate and air-dried. Spot 5 μL each of the test solution and the standard solution on the plate. Develop the plate with the upper layer of a mixture of ethyl acetate and ammonia solution (28) (200 : 9) to a distance of about 10 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and the spot of the starting point from the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.5 % (0.5 g, in vacuum, 60 °C, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.5 g of Tulobuterol Hydrochloride, previously dried, dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titrration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 26.419 mg of $C_{12}H_{18}ClNO \cdot HCl$

Containers and Storage ***Containers***—Tight containers.

Tyrothricin

Tyrothricin consists principally of gramicidin and tyrocidine.
Tyrothricin contains not less than 900 μg (potency) and not more than 1400 μg (potency) per mg of tyrothricin.

Description Tyrothricin appears as white to brownish white powder, is odorless or almost odorless, and is almost tasteless.
Tyrothricin is soluble in ethanol (95), freely soluble in acetic acid (100), slightly soluble in acetone, and practically insoluble in water, in chloroform, or in ether.

Identification To 5 mg (potency) of Tyrothricin add 5 mL of 4-dimethylaminobenzaldehyde-iron (III) chloride TS, shake well for 2 minutes, and add 2 drops of 0.1 mol/L sodium nitrite and 5 mL of water: a blue color develops.

Purity ***Fat***—Weigh accurately about 1.0 g of Tyrothricin, previously dried at 105 °C for 3 hours, mix with 2 g of asbestos, previously ignited and washed with hexane, transfer to a thimble filter for extraction inside a Soxhlet extractor, extract with hexane for 18 hours using a 85 to 100 mL extraction flask, remove the extraction flask, evaporate the solvent in a water bath, dry at 105 °C for 2 hours, cool, and weigh the mass: the fat content is the difference in the mass before and after the procedure (not more than 6.0 %).

Loss on Drying Not more than 5.0 % (0.1 g, 0.7 kPa, 60 °C, 3 hours).

Residue on Ignition Not more than 3.5 % (1 g).

Assay (1) Culture medium- Agar medium for transferring the test organism

Peptone	5.0 g
Potassium dihydrogen phosphate	2.0 g
Yeast extract	20.0 g
Polysorbate 80	0.1 g
Glucose	100 g
Agar	15.0 ~ 20.0 g
Purified water	1000 mL

Mix all the components, and sterilize. Adjust the pH so that it will be 6.7 to 6.8 after sterilization.

(2) Liquid medium for suspending the test organism

Peptone	5.0 g
Glucose	1.0 g
Yeast extract	1.5 g
Dipotassium hydrogen phosphate	3.68 g
Beef extract	1.5 g
Potassium dihydrogen phosphate	1.32 g
Sodium chloride	3.5 g

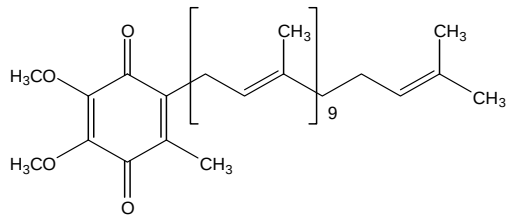
Mix all the components, and sterilize. Adjust the pH so that it will be 6.95 to 7.05 after sterilization.

(3) Test organism and test organism suspension- Use *Streptococcus faecium* ATCC 10541 as the test organism. Puncture the test organism in the agar medium for transferring the test organism, subculture not less than 3 times at 37 °C for 20 to 24 hours, and keep at a temperature not exceeding 5 °C. Transfer the organism so obtained to 8 to 10 mL of the liquid medium for suspending the test organism, incubate at 37 °C for 20 to 24 hours, and use as the bacterial suspension. Before use, add 1.0 mL of this bacterial suspension to 100 mL of the liquid medium for suspending the test organism, and use as the test organism suspension.

(4) Weigh accurately a suitable amount of Tyrothricin, dissolve in ethanol (95) to make a solution of suitable concentration, dilute with ethanol so that each mL contains 0.200 μg (potency), and use this solution as the test solution. Separately, weigh accurately a suitable amount of Gramicidin RS, add ethanol (95) so that each mL contains 1 mg (potency), and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 5 °C, and use within 30 days. Pipet a suitable volume of the standard stock solution, dilute with ethanol so that each mL contains 0.028, 0.034, 0.040, 0.048, and 0.057 μg (potency), and use these solutions as the standard solutions. Put 1.0 mL each of the standard solutions and test solution into test tubes for each group. Calculate the average absorbance of the test solution, calculate the concentration of gramicidin from the standard curve of gramicidin, and multiply this figure by 5 to calculate the amount (μg) of tyrothricin in the sample.

Containers and Storage ***Containers***—Tight containers.

Ubidecarenone



$C_{59}H_{90}O_4$: 863.34

2-[(2*E*,6*E*,10*E*,14*E*,18*E*,22*E*,26*E*,30*E*,34*E*)-3,7,11,15,19,23,27,31,35,39-Decamethyltetraconta-2,6,10,14,18,22,26,30,34,38-decaenyl]-5,6-dimethoxy-3-methylcyclohexa-2,5-diene-1,4-dione [*303-98-0*]

Ubidecarenone contains not less than 98.0 % and not more than 101.0 % of ubidecarenone ($C_{59}H_{90}O_4$), calculated on the anhydrous basis.

Description Ubidecarenone is a yellow to orange crystalline powder, is odorless and has no taste.
Ubidecarenone is soluble in ether, very slightly soluble in ethanol (99.5) and practically insoluble in water.
Ubidecarenone is gradually colored by light.

Melting point—About 48 °C.

Identification (1) Dissolve 50 mg of Ubidecarenone in 1 mL of ether and add 10 mL of ethanol (99.5). To 2 mL of this solution, add 3 mL of ethanol (99.5) and 2 mL of dimethyl malonate, then add dropwise 1 mL of a solution of potassium hydroxide (1 in 5) and mix: a

blue color is observed.

(2) Determine the infrared spectra of Ubidecarenone and Ubidecarenone RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Ubidecarenone according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Dissolve 50 mg of Ubidecarenone in 50 mL of ethanol (99.5) by warming at about 50 °C for 2 minutes, cool and use this solution as the test solution. To exactly 1 mL of the test solution, add ethanol (99.5) to make exactly 100 mL and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of peaks other than the principal peak from the test solution is not larger than the principal peak area from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, flow rate and selection of column: Proceed as directed in the operating conditions in the Assay.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of Ubidecarenone obtained from 5 μL of the standard solution is between 20 mm and 40 mm.

Time span of measurement: About twice of the retention time of Ubidecarenone after the solvent peak.

Water Not more than 0.2 % (1 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 50 mg of Ubidecarenone and Ubidecarenone RS (previously determine the water content), dissolve each in 40 mL of ethanol (99.5) by warming at about 50 °C for 2 minutes, cool, add ethanol (99.5) to make exactly 50 mL each and use this solution as the test solution and the standard solution, respectively. Perform the test with 5 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions and determine peak areas, A_T and A_S, of Ubidecarenone of for the test solution and the standard solution, respectively.

$$\text{Amount (mg) of ubidecarenone } (C_{59}H_{90}O_4) = \text{Amount (mg) of Ubidecarenone RS, calculated on the anhydrous basis} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 275 nm).

Column: A stainless steel column, about 5 mm in internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35 °C.

Mobile phase: A mixture of methanol and ethanol (99.5) (13 : 7).

Flow rate: Adjust the flow rate so that the retention time of Ubidecarenone is about 10 minutes.

System suitability

System performance: Dissolve each 10 mg of Ubidecarenone and ubiquinone-9 in 20 mL of ethanol (99.5) by warming at about 50 °C for 2 minutes. After cooling, when the procedure is run with 5 μL of this solution under the above operating conditions, ubiquinone-9 and ubidecarenone are eluted in this order with the resolution between their peaks being not less than 4.0.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ubidecarenone is not more than 0.8 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Undecylenic Acid

$C_{11}H_{20}O_2$: 184.28

Undec-10-enoic acid [*112-38-9*]

Undecylenic Acid contains not less than 97.0 % and not more than 100.5 % of undecylenic acid ($C_{11}H_{20}O_2$).

Description Undecylenic Acid is a colorless or pale yellow color liquid and has characteristic odor.

Undecylenic Acid is miscible with ethanol (95), with ether, with chloroform, with benzene or with fixed and volatile oils.

Undecylenic acid is practically insoluble in water.

Identification (1) To 1 mL of Undecylenic Acid, add 1 mL of potassium permanganate TS dropwise: the permanganate color disappears.

(2) Place 3 mL of Undecylenic Acid and 3 mL of freshly distilled aniline and heat for 10 minutes with reflux condenser. After cooling, add 10 mL of ethanol

(95) and 10 mL of ether and transfer to a separatory funnel. Wash the ether solution with four 20 mL volumes of water and discard the water washings. Heat in a steam-bath until the odor of ether no longer is perceptible, then a portion of activated carbon, mix and filter. Evaporate the filtrate to dryness and recrystallize the residue from 70 % ethanol: it melts between 66 °C and 67.6 °C.

Refractive Index n_D^{25} : 1.447 ~ 1.448.

Specific Gravity d_{25}^{25} : 0.910 ~ 0.913.

Iodine Value 131 ~ 138.

Congealing Point Not lower than 21 °C.

Purity (1) ***Water soluble acids***—Shake 5 mL of Undecylenic Acid with 5 mL of water and filter the water layer through a filter paper previously moistened with water. Add 1 drop of methyl orange and titrate with 0.01 mol/L sodium hydroxide: not more than 1.0 mL of 0.01 mol/L sodium hydroxide is required to match the color produced by 1 drop of methyl orange in 5 mL of water.

(2) ***Heavy metals***—Proceed with 1.0 g of Undecylenic Acid according to Method 2 and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

Residue on Ignition Not more than 0.15 % (3 g).

Assay Weigh accurately about 0.75 g of Undecylenic Acid, dissolve in 50 mL of ethanol (95), add 3 drops of phenolphthalein TS and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). The end point of the titration is only when a pale red color persists for not less than 30 seconds. Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 18.428 mg of $C_{11}H_{20}O_2$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Urokinase

[*62229-50-9*]

Urokinase is an enzyme, obtained from human urine, that activates plasminogen and has the molecular weight of about 54000. Urokinase is a solution using a suitable buffer solution as the solvent.
Urokinase contains not less than 60000 units per mL and not less than 120000 units per mg of protein.

Description Urokinase is a clear, colorless liquid.

pH—5.5 ~ 7.5.

Identification (1) Dissolve 70 mg of fibrinogen in 10 mL of phosphate buffer solution, pH 7.4, add 1 mL of solution of thrombin containing 10 units per mL in Isotonic Sodium Chloride Injection, mix, place in a Petri dish about 90 mm in internal diameter and keep horizontally until the solution is coagulate. On the surface, drop 10 μL of Urokinase containing 100 units per mL in gelatin-tris buffer solution and stand overnight: lysis circle is appeared.

(2) Dissolve 1.0 g of powdered agar in 100 mL of boric acid-sodium hydroxide buffer solution, pH 8.4, by warming and pour the solution into a Petri dish until the height comes to about 2 mm. After cooling, make two wells of 2.5 mm in diameter with the space of 6 mm. To each well, place separately 10 μL of Urokinase containing 30000 units per mL in Isotonic Sodium Chloride Injection and 10 μL of anti-urokinase serum and stand overnight: a clear precipitated line is appeared.

Purity (1) ***Heavy metals*** —Proceed with 2.0 mL of Urokinase according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Blood group substances*** —Dilute Urokinase with Isotonic Sodium Chloride Injection so that each mL of the solution contains 12000 units and use this solution as the test solution. To anti-A type antibody for blood typing, add Isotonic Sodium Chloride Injection to dilute each 32, 64, 128, 256, 512 and 1024 times, place separately 25 μL each of these solutions in six wells on the first and second lane of a V-shaped 96-well microplate. Next, add 25 μL of the test solution into the six wells on the first lane and 25 μL of Isotonic Sodium Chloride Injection into the six wells of the second lane, mix and allow to stand for 30 min. To each well, add 50 μL of A-type erythrocyte suspension, mix, allow to stand for 2 hours and compare the agglutination of erythrocyte in both lanes: dilution factor of anti-A type antibody of the wells which show the agglutination is equal in both lanes. Perform the same test with anti-A type antibody for blood typing and A-type erythrocyte suspension.

Abnormal Toxicity Dilute Urokinase with Isotonic Sodium Chloride Injection so that each mL of the solution contains 12000 units and use this solution as the test solution. Inject 5.0 mL of the test solution into the peritoneal cavity of each of 2 or more of well-nourished, healthy guinea pigs weighing about 350 g and inject 0.5 mL of the test solution into peritoneal cavity of each 2 or more of well-nourished, healthy mice aged about 5 weeks. Observe the conditions of the animals for more than 7 days: all the animals exhibit no abnormalities.

High Molecular Mass Urokinase Dilute Urokinase with gelatin-phosphate buffer solution so that each mL of the solution contains 10000 units and use this solution as the test solution. Perform the test with 100 μL of the test solution as directed under Liquid Chromatography according to the following conditions. Determine the areas of two peaks eluted closely at about 35 minutes having smaller retention time, A_a and larger retention time, A_b, by the automatic integration method: the value, $A_a/(A_a+A_b)$, is not less than 0.85.

Operating conditions

Apparatus: Use a pumping system for the mobile phase, a sample injection port, a column, a pumping system for the reaction reagent, a reaction coil, a reaction chamber, a spectrofluorometer and a recorder. Attach a 3-way tube to the outlet for the mobile phase of the column, connect the pumping system for the reaction reagent and the reaction coil, and join outlet of the reaction coil to the spectrofluorometer.

Detector: Spectrofluorometer (excitation wavelength: 365 nm, fluorescence wavelength: 460 nm).

Column: A stainless steel column, 7.5 mm in internal diameter and 60 cm in length, packed with porous silica gel for liquid chromatography (10 μm to 12 μm in particle diameter).

Column temperature: A constant temperature of about 20 °C.

Reaction coil: A stainless steel column, 0.25 mm in internal diameter and 150 cm in length.

Reaction coil temperature: 37 °C.

Mobile phase: Gelatin-phosphate buffer solution.

Flow rate of mobile phase: 0.5 mL/minute.

Reaction reagent: 7-(Glutarylglycyl-L-arginylamino)-4-methylcoumarin TS.

Flow rate of reaction reagent: 0.75 mL/minute.

Selection of column: Adjust the pH of Urokinase to 7.5 with sodium hydroxide TS, allow to stand at 37 °C for over 24 hours and add gelatin-phosphate buffer solution to make the solution containing 20000 units per mL. Proceed with 100 μL of this solution under the above operating conditions and calculate the resolution. Use a column giving elution of high molecular weight Urokinase (54000), low molecular weight Urokinase (33000) in this order with the resolution between their peaks being not less than 1.0.

Assay (1) ***Urokinase*** —Pipet 1.0 mL of Urokinase, dilute exactly with gelatin-tris buffer solution so that each mL of the solution contains about 30 units and use this solution as the test solution. Add exactly 2 mL of gelatin-tris buffer solution to contents of one ample of high molecular weight Urokinase RS to dissolve, pipet 1.0 mL of this solution, dilute exactly with gelatin-tris buffer solution so that each mL of the solution contains about 30 units and use this solution as the standard solution. Place 1.0 mL of L-pyrglutamyl-glycyl-L-arginine-*p*-nitroaniline hydrochloride TS in two silicon-coated test tubes, about 10 mm in internal diameter, warm in a water-bath at 35 ± 0.2 °C for 5 minutes, add separately 0.50 mL each the test solution and the standard solution, warm in a water-bath at 35 ± 0.2 °C for exactly 30 minutes and then add 0.50 mL of diluted acetic acid (100) (2 in 5). Determine the absorbances, A_T and A_S, of these solutions at 405 nm as directed under Ultraviolet-visible Spectrophotometry, using water as the blank. Separately, place 1.0 mL of L-pyroglutamylglycyl-L-arginine-*p*-nitroaniline hydrochloride TS in two test tubes, add 0.50 mL of diluted acetic acid (100) (2 in 5) and 0.50 mL each of the test solution and the standard solution. Determine the absorbances, A_{T0} and A_{S0}, of these solutions at 405 nm as the same manner, using water as the blank.

$$\text{Amount (units) of Urokinase} = \frac{A_T - A_{T0}}{A_S - A_{S0}} \times a \times b$$

a: Amount (units) of Urokinase in 1 mL of the standard solution,

b: Total volume (mL) of the test solution.

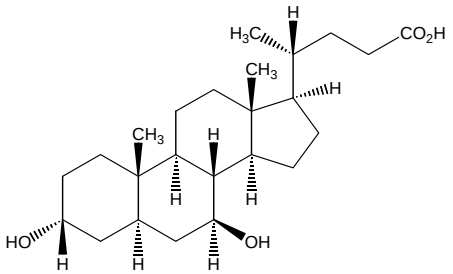
(2) ***Protein*** —Measure exactly a volume of Urokinase, equivalent to about 15 mg of protein and perform the test as directed under the Nitrogen Determination.

Each mL of 0.005 mol/L sulfuric acid VS
= 0.87544 mg of protein

Containers and Storage ***Containers***—Tight containers.

Storage—Not exceeding -20 °C.

Ursodeoxycholic Acid



Ursodesoxycholic Acid $C_{24}H_{40}O_4$: 392.57

(4R)-4-[(1*S*,2*S*,5*R*,7*S*,9*S*,10*R*,11*S*,14*R*,15*R*)-5,9-Dihydroxy-2,15-dimethyltetracyclo [8.7.0.0^{2,7}.0^{11,15}]heptadecan-14-yl] pentanoic acid [*128-13-2*]

Ursodeoxycholic Acid, when dried, contains not less than 98.5 % and not more than 101.0 % of ursodeoxycholic acid ($C_{24}H_{40}O_4$).

Description Ursodeoxycholic Acid appears as white crystals or powder, and has a bitter taste.

Ursodeoxycholic Acid is freely soluble in methanol, in ethanol (99.5), or in acetic acid (100), and practically insoluble in water.

Identification (1) Dissolve 10 mg of Ursodeoxycholic Acid, add 1 mL of sulfuric acid and 1 drop of formaldehyde VS and allow to stand for 5 minutes. To the solution, add 5 mL of water: a blue-green suspended substance is produced.

(2) Determine the infrared spectra of Ursodeoxycholic Acid and Ursodeoxycholic Acid RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +59.0 ~ +62.0° (after drying, 1.0 g, ethanol (99.5), 25 mL, 100 mm).

Melting Point 200 ~ 204 °C.

Purity (1) ***Odor***—To 2.0 g of Ursodeoxycholic Acid, add 100 mL of water and boil for 2 minutes: no odor is perceptible.

(2) ***Chloride***—Dissolve 2.0 g of Ursodeoxycholic Acid in 20 mL of acetic acid (100) with shaking, add water to make 200 mL, shake thoroughly and allow to stand for 10 minutes. Filter this solution, discard the first 10 mL of the filtrate and use the subsequent filtrate as the test solution. To 40 mL of the test solution, add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.25 mL of hydrochloric acid, add 4 mL of acetic acid (100), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.022 %).

(3) ***Sulfate***—To 40 mL of the test solution obtained in (2), add 1 mL of dilute hydrochloric acid and water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS, add 4 mL of acetic acid (100), 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048 %).

(4) ***Heavy metals***—Proceed with 1.0 g of Ursodeoxycholic Acid according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(5) ***Barium***—To the solution obtained in (1), add 2 mL of hydrochloric acid, boil for 2 minutes, cool, filter and wash with water until the last washing makes 100 mL. To 10 mL of the solution, add 1 mL of dilute sulfuric acid: no turbidity is produced.

(6) ***Arsenic***—Prepare the test solution with 1.0 g of Ursodeoxycholic Acid according to Method 3 and perform the test (not more than 2 ppm).

(7) ***Related substances***—Dissolve 0.20 g of Ursodeoxycholic Acid in 1 mL of methanol, add acetone to make exactly 10 mL, and use this solution as the test solution. To 1 mL of this solution add acetone to make exactly 100 mL. To 1 mL and 2 mL of this solution add acetone to make exactly 20 mL, and use these solutions as the standard solution (1) and standard solution (2), respectively. Separately, dissolve 50 mg of Chenodeoxycholic Acid RS in 5 mL of methanol, and add acetone to make exactly 50 mL. To 2 mL of this solution add acetone to make exactly 10 mL, and use this solution as the standard solution (3). Dissolve 25 mg of Lithocholic Acid RS in 5 mL of methanol, and add acetone to make exactly 50 mL. To 2 mL of this solution add acetone to make exactly 50 mL, and use this solution as the standard solution (4). Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and standard solutions (1), (2), (3), and (4) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of isooctane, ethanol (95), ethyl acetate, and acetic acid (100) (10 : 2 : 7 : 1) to a distance of about 10 cm, and air-dry the plate. Dry at 120 °C for 30 minutes, and immediately spray evenly a solution prepared by dissolving 5 g of phosphomolybdic acid *n*-hydrate in ethanol (99.5) to make 50 mL, to which 5 mL of sulfuric acid is dropped in and ethanol (99.5) is added to make 100 mL, and heat at 120 °C for 3 to 5 minutes: the spot from the test solution is not more intense than the spots from the standard solutions (3) and (4), and the spots other than the principal spot and other than the spots mentioned above from the test solution are not more intense than the spot from the standard solution (2). The total amount of the spots other than the principal spot and other than the spots mentioned above from the test solution, which is calculated by comparison with the sopts obtained from the standard solutions (1) and (2), is not more than 0.25 %.

Loss on Drying Not more than 1.0 % (1 g, 105 °C, 2 hours).

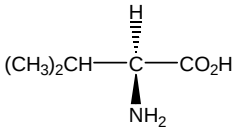
Residue on Ignition Not more than 0.2 % (1 g).

Assay Weigh accurately about 0.5 g of Ursodeoxycholic Acid, previously dried and dissolve in 40 mL of ethanol (95) and 20 mL of water. Titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 39.26 mg of $C_{24}H_{40}O_4$

Containers and Storage ***Containers***—Well-closed containers.

L-Valine



$C_5H_{11}NO_2$: 117.15

(2*S*)-2-Amino-3-methylbutanoic acid [*72-18-4*]

L-Valine, when dried, contains not less than 98.5 % and not more than 101.0 % of L-valine ($C_5H_{11}NO_2$).

Description L-Valine appears as white crystals or crystalline powder, is odorless or has a faint characteristic odor and has a slightly sweet taste, which becomes bitter.
L-Valine is freely soluble in formic acid, soluble in water and practically insoluble in ethanol (95) or in ether.
L-Valine dissolves in dilute hydrochloric acid.

Identification Determine the infrared spectra of L-Valine and L-Valine RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific Optical Rotation $[\alpha]_D^{20}$: +26.5 ~ + 29.0° (after drying, 2 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH Dissolve 0.5 g of L-Valine in 20 mL of water: the pH of this solution is between 5.5 and 6.5.

Purity (1) ***Clarity and color of solution***—Dissolve 0.5 g of L-Valine in 20 mL of water: the solution is clear and colorless.

(2) ***Chloride***— Proceed with 0.5 g of L-Valine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021 %).

(3) ***Sulfate***— Proceed with 0.6 g of L-Valine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028 %).

(4) ***Ammonium***— Proceed with 0.25 g of L-Valine. Prepare the control solution with 5.0 mL of standard ammonium solution (not more than 0.02 %).

(5) ***Heavy metals***—Proceed with 2.0 g of L-Valine according to Method 1 under Heavy Metals Limit Test and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 15 ppm).

(6) ***Iron***—Dissolve 0.333 g of L-Vaine in water to make 45 mL, add 2 mL of hydrochloric acid, and use this solution as the test solution. To 1.0 mL of standard iron solution add water to make 45 mL, add 2 mL of hydrochloric acid, and use this solution as the standard solution. To the test solution and standard solution add 50 mg of ammonium peroxydisulfate and 3 mL of 30 % ammonium thiocyanate, and mix: the color of the test solution is not more intense than that of the standard solution (not more than 30 ppm).

(6) ***Arsenic***—Prepare the test solution with 1.0 g of L-Valine according to Method 2 and perform the test (not more than 2 ppm).

(7) ***Related substances***—Dissolve 0.10 g of L-Valine in 25 mL of water and use this solution as the test solution. Pipet 1.0 mL of the test solution and add water to make exactly 50 mL. Pipet 5.0 mL of this solution, add water to make exactly 20 mL and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3 : 1 : 1) to a distance of about 10 cm and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate and heat at 80 °C for 5 minutes: the spots other than the principal spot form the test solution are not more intense than the spot from the standard solution.

Loss on Drying Not more than 0.3 % (1 g, 105 °C, 3 hours).

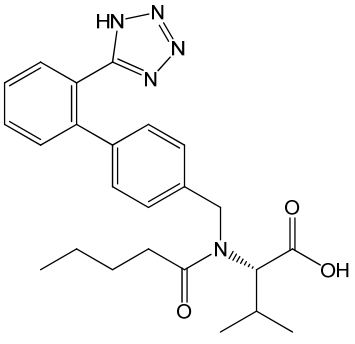
Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.12 g of L-Valine, previously dried and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 11.715 mg of $C_5H_{11}NO_2$

Containers and Storage ***Containers***—Tight containers.

Valsartan



$C_{24}H_{29}N_5O_3$: 435.52

(*S*)-3-Methyl-2-[pentanoyl-[[4-[2-(2*H*-tetrazol-5-yl)phenyl]phenyl] methyl]amino]butanoic acid
[*137862-53-4*]

Valsartan contains not less than 98.0 % and not more than 102.0 % of valsartan ($C_{24}H_{29}N_5O_3$), calculated on the anhydrous basis.

Description Valsartan appears as white powder.
Valsartan is freely soluble in ethanol(95), sparingly soluble in dichloromethane, and practically insoluble in water.
Valsartan is hygroscopic.

Identification (1) Determine the infrared spectra of Valsartan and Valsartan RS, as directed in the paste method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) The retention time of the principal peak in the chromatogram of the test solution corresponds to that in the chromatogram of the standard solution, as obtained in the Assay.

Purity (1) ***Clarity and color of solution***—Dissolve 1.0 g of Valsartan in 20 mL of methanol, and perform the test as directed under Ultraviolet-visible Spectrophotometry: the value of the absorbance at 420 nm divided by the path length is not more than 0.02.

(2) ***Heavy metals***—Proceed with 1.0 g of Valsartan according to Method 2 and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—(i) Valsartan related substance I- Weigh accurately about 50 mg of Valsartan, add 40 mL of the mobile phase, sonicate for 5 minutes to dissolve, add the mobile phase to make exactly 50mL, and use this solution as the test solution. Separately, weigh a suitable amount of Valsartan Related Substance I {(*R*-*N*-valeryl-*N*-([2'-(1*H*-tetrazole-5-yl)biphen-4-yl] methyl)valine} RS, add the mobile phase to make a solution so that each mL contains 0.01 mg, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according the following operating conditions. Determine each peak area of each solution by the automatic integration method, and calculate the amount of valsartan related substance I according to the following equation: not more than 1.0 %.

$$\text{Amount (\%) of valsartan related substance I} = 100 \times \frac{C_S}{C_T} \times \frac{A_T}{A_S}$$

C_T: Concentration (mg/mL) of Valsartan in the test solution

C_S: Concentration (mg/mL) of valsartan related substance I in the standard solution

A_T: Peak area of valsartan related substance I in the test solution

A_S: Peak area of valsartan related substance I in the standard solution

Operation conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with cellulose tris-3,5-dimethylphenylcarbamate coated porous silica gel for liquid chromatography (5 μm in particle diameter)

Mobile phase: A mixture of hexane and 2-propanol and trifluroactic acid (82 : 15: 0.1).

Flow rate: 0.8 mL/minute.

System suitability

System performance: When the procedure is run with 10 μL of the system suitability solution under the above operating conditions, the resolution between the peaks of valsartan and valsartan related substance I is not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL each of the system suitability solution under the above operating conditions, the relative standard deviation of the peak area of valsartan related substance I is not more than 5.0 %.

System suitability solution—Weigh accurately a sutible amount of Valsartan RS and Valsartan Related Substance I RS, and dissolve in the mobile phase to make a solution so that each mL contains 0.04 mg each.

(ii) Valsartan related substance II, Valsartan related substance III and other related substances- Weigh accurately about 50 mg of Valsartan, add the mobile phase to make exactly 100 mL and use this solution as the test solution. Separately, weigh accurately a suitable amount each of Valsartan Related Substance II {*S*-*N*-butyryl-*N*-([2'-(1*H*-tetrazole-5-yl)biphen-4-yl]methyl)-valine} RS and Valsartan Related Substance III {*S*-*N*-valeryl-*N*-([2'-(1*H*-tetrazole-5-yl) biphen-4-yl]methyl)-valine benzyl ester} RS, add the mobile phase to make a solution so that each mL contains 0.001 mg each, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under Liquid Chromatography according the following operating conditions. Determine each peak area of each solution by the automatic integration method, and calculate the amount of related substances: the amount of valsartan related substance II is not more than 0.2 %; the amount of valsartan related substance III is not more than 0.1 %; the amount of each other related substance except valsartan related substance I is not more than 0.1 %; the total amount of all the related substances except valsartan related substance I is not more than 0.3 %.

Amount (%) of valsartan related substance

$$= 100 \times \frac{C_S}{C_T} \times \frac{A_i}{A_S}$$

C_T: Concentration (mg/mL) of Valsartan in the test solution

C_S: Concentration (mg/mL) of each valsartan related substance in the standard solution (concentration (mg/mL) of each Valsartan Related Substance RS in the standard solution)

A_i: Peak area of each valsartan related substance in the test solution

A_S: Peak area of each valsartan related substance in the standard solution

Operation conditions

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column, mobile phase and, flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the resolution between the peaks of valsartan and valsartan related substance II is not less than 1.8.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviations of the peak area of valsartan related substance II and valsartan are not more than 10.0 % and not more than 2.0 %, respectively.

Water Not more than 2.0 % (1 g, volumetric titration, direct titration).

Loss on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 50 mg each of Valsartan and Valsartan RS, dissolve in the mobile phase to make exactly 100 mL, and use these solutions as the test solution and standard solution. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of valsartan for the test solution and standard solution, respectively.

$$\text{Amount (mg) of valsartan } (C_{24}H_{29}N_5O_3) = \text{Amount (mg) of Valsartan RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 273 nm).

Column: A stainless steel column about 3.0 mm in internal diameter and about 125 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 mm in particle diameter).

Mobile phase: Prepare a filtered and degassed mixture of water and acetonitrile, and acetic acid (100) (500 : 500 : 1).

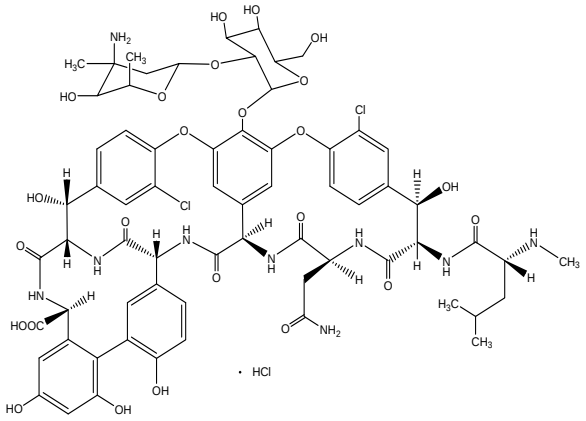
Flow rate: 4 mL/min.

System suitability

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of Valsartan is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

Vancomycin Hydrochloride



$C_{66}H_{75}Cl_2N_9O_{24}$·HCl: 1485.72

(1*S*,2*R*,18*R*,19*R*,22*S*,25*R*,28*R*,40*S*)-50-[3-Amino-2,3,6-trideoxy-3-C-methyl-α-L-*lyxo*-hexopyranosyl-(1→2)-β-D-glucopyranosyloxy]-22-carbamoylmethyl-5,15-dichloro-2,18,32,35,37-pentahydroxy-19-[(2*R*)-4-methyl-2-(methylamino)pentanoylamino]-20,23,26,42,44-pentaoxo-7,13-dioxa-21,24,27,41,43-pentaazaoctacyclo[26.14.2.2^{3,6}.2^{14,17}.1^{8,12}.1^{29,33}.0^{10,25}.0^{34,39}]pentaconta-3,5,8,10,12(50),14,16,29,31,33(49),34,36,38,45,47-pentadecaene-40-carboxylic acid monohydrochloride [*1404-93-9*]

Vancomycin Hydrochloride is the hydrochloride of a glycopeptides substance having antibacterial activity produced by the growth of *Streptomyces orientalis*.

Vancomycin Hydrochloride contains not less than 1000 μg (potency) and not more than 1200 μg (potency) per mg of vancomycin ($C_{66}H_{75}Cl_2N_9O_{24}$: 1449.25), calculated on the anhydrous basis.

Description Vancomycin Hydrochloride appears as white powder.

Vancomycin Hydrochloride is freely soluble in water, soluble in formamide, slightly soluble in methanol,

very slightly soluble in ethanol (95), and practically insoluble in acetonitrile.
Vancomycin Hydrochloride is hygroscopic.

Identification (1) Determine the absorption spectra of solutions of Vancomycin Hydrochloride and Vancomycin Hydrochloride RS in water (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Vancomycin Hydrochloride and Vancomycin Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Weigh about 20 mg of Vancomycin Hydrochloride, dissolve in 10 mL of water, and add one drop of silver nitrate TS: the solution turns to turbid.

Specific Optical Rotation $[\alpha]_D^{20}$: -30 ~ -40° (0.2 g calculated on the anhydrous basis, water, 20 mL, 100 mm)

pH The pH of a solution obtained by dissolving 0.25 g of Vancomycin Hydrochloride in 5 mL of water is between 2.5 and 4.5.

Purity (1) ***Heavy metals***—Proceed with 1 g of Vancomycin Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 20 ppm).

(2) ***Related substances***—Dissolve 0.1 g of Vancomycin Hydrochloride in 10 mL of the mobile phase A, and use this solution as the test solution. Pipet 1 mL of the test solution, add the mobile phase A to make exactly 25 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions. If necessary, proceed with 20 μL of mobile phase A in the same manner to compensate for the baseline. Determine the peak areas of the test solution and standard solution by the automatic integration method: the area of each peak other than the peak of vancomycin from the test solution is not larger than the peak area of vancomycin from the standard solution, and the total area of the peaks other than vancomycin from the test solution is not larger than 3 times the peak area of vancomycin from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 280 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Control the step or concentration gradient by mixing the mobile phases A and B as directed in the following table.

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-12	100	0
12-20	100→30	0→100
20-22	0	100

Mobile phase A: To 4 mL of triethylamine add water to make 2000 mL. Adjust the pH to 3.2 with phosphoric acid. To 920 mL of this solution add 70 mL of acetonitrile and 10 mL of tetrahydrofuran. Adjust the amount of acetonitrile so that the retention time of vancomycin is 7.5 to 10.5 minutes.

Mobile phase B: To 4 mL of triethylamine add water to make 2000 mL. Adjust the pH to 3.2 with phosphoric acid. To 700 mL of this solution add 290 mL of acetonitrile and 10 mL of tetrahydrofuran.

Flow rate: 1.5 mL/minute

System suitability

Detection sensitivity: The peak area of vancomycin obtained from 20 μL of the standard solution is equivalent to 3 to 5 % of that from 20 μL of the test solution.

System performance: Dissolve 5 mg of Vancomycin Hydrochloride in 10 mL of water, warm at 65 °C for 48 hours, and cool at ordinary temperature. When the procedure is run with 20 μL of this solution under the above operating conditions, related substance I and related substance II are eluted in this order. The resolution between the peaks of related substance I and vancomycin is not less than 3, the number of theoretical peaks of the peak of vancomycin is not less than 1500, and related substance II is eluted between 15 and 18 minutes.

System repeatability: When the test is repeated 5 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of vancomycin is not more than 2.0 %.

Time span of measurement: About 2.5 times as long as the retention time of vancomycin beginning after the solvent peak

Water Not more than 5.0 % (0.1 g, volumetric titration, direct titration). Use a mixture of formamide for water determination and methanol for water determination (3 : 1).

Residue on Ignition Not more than 1.0 % (1.0 g)

Sterility Test It meets the requirement, when Vancomycin Hydrochloride is used in a sterile preparation.

Bacterial Endotoxins Less than 0.25 EU/mg of vancomycin, when Vancomycin Hydrochloride is used in a sterile preparation.

Assay ***The Cylinder-plate method*** (1) Culture medium (i) Agar media for seed and base layer-

Peptone	5.0 g
Meat extract	3.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.2 to 6.4 after sterilization.

(2) Test organism- *Bacillus subtilis* ATCC 6633

(3) Weigh accurately about 25 mg (potency) of Vancomycin Hydrochloride and dissolve in water to make exactly 25 mL. Pipet a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make solutions so that each mL contains 100.0 μg (potency) and 25.0 μg (potency), and use these solutions as the high concentration test solution and the low concentration test solution, respectively. Separately, weigh accurately about 25 mg (potency) of Vancomycin Hydrochloride RS, dissolve in water to make exactly 25 mL, and use this solution as the standard stock solution. Keep the standar stock solution at 5 °C or below, and use within 7 days. Take exactly a suitable amount of the standard stock solution, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make solutions so that each mL contains 100.0 μg (potency) and 25.0 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively. Perform the test with these solutions as directed in I) (8) under Microbial Assay for Antibiotics.

Containers and Storage ***Containers***—Tight containers.

Vancomycin Hydrochloride Capsules

Vancomycin Hydrochloride Capsules are capsules of Vancomycin Hydrochloride dispersed in polyethylene glycol. Vancomycin Hydrochloride Capsules contain not less than 90.0 % and not more than 120.0 % of the labeled amount of vancomycin ($C_{66}H_{75}Cl_2N_9O_{24}$: 1449.26).

Method of Preparation Prepare as directed under Capsules, with Vancomycin Hydrochloride.

Identification Dissolve an amount of the contents of Vancomycin Hydrochloride Capsules, equivalent to 10 mg of vancomycin hydrochloride, in water to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 279 and 284 nm.

Water Not more than 8.0 % (0.2 g, volumetric titration, direct titration)

Dissolution Test Perform the test with 1 capsule of Vancomycin Hydrochloride Capsules at 100 revolutions per minute according to Method 2 under Dissolution Test, using 900 mL of water as the dissolution solution. Take the dissolved solution 45 minutes after the start of the test, filter, use the filtrate as the test solution, and perform the test as directed in the Assay under Vancomycin Hydrochloride. The dissolution rate of Vancomycin Hydrochloride Capsules in 45 minutes is not less than 85 %.

Uniformity of Dosage Units It meets the requirement.

Assay ***The Cylinder-plate method*** Perform the test as directed in the Assay under Vancomycin Hydrochloride. Weigh accurately the contents of not less than 20 Vancomycin Hydrochloride Capsules. Weigh accurately an amount of the contents, equivalent to about 25 mg (potency) of Vancomycin Hydrochloride according to the labeled potency, and dissolve in water to make exactly 25 mL. Pipet a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make solutions so that each mL contains 100 μg (potency) and 25 μg (potency), and use these solutions as the high concentration test solution and the low concentration test solution, respectively.

Containers and Storage ***Containers***—Tight containers.

Vancomycin Hydrochloride for Injection

Vancomycin Hydrochloride for Injection is a preparation for injection which is dissolved before use.
Vancomycin Hydrochloride for Injection contains not less than 90.0 % and not more than 120.0 % of the labeled amount of vancomycin ($C_{66}H_{75}Cl_2N_9O_{24}$: 1449.25).

Method of Preparation Prepare as directed under Injection, with Vancomycin Hydrochloride.

Description Vancomycin Hydrochloride for Injection appears as white masses or powder.

Identification (1) Dissolve an amount of Vancomycin Hydrochloride for Injection, equivalent to 5 mg (potency) of vancomycin hydrochloride according to the labeled amount, in 50 mL of water, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometery: it exhibits a maximum between 279 and 283 nm.

(2) Dissolve an amount of Vancomycin Hydrochlo-

ride for Injection, equivalent to 20 mg (potency) of vancomycin hydrochloride, in 10 mL of water, and add 1 drop of silver nitrate TS: a white turbidity is produced.

pH The pH of a solution prepared by dissolving an amount of Vancomycin Hydrochloride for Injection, equivalent to 0.5 g (potency) of vancomycin hydrochloride according to the labeled amount, in 10 mL of water is between 2.5 and 4.5.

Purity (1) ***Clarity and color of solution***—Dissolve an amount of Vancomycin Hydrochloride for Injection, equivalent to 0.5 g (potency) of vancomycin hydrochloride according to the labeled amount, in 10 mL of water: the solution is clear and colorless or pale yellow, and the absorbance of the solution, determined at 465 nm as directed under Ultraviolet-visible Spectrophotometry, is not more than 0.05.

(2) ***Heavy metals***—Proceed with 0.66 g of Vancomycin Hydrochloride for Injection according to Method 2 and perform the test. Prepare the control solution with 3.0 mL of standard lead solution (not more than 30 ppm).

(3) ***Related substances***—Dissolve an amount of Vancomycin Hydrochloride for Injection, equivalent to 0.1 g (potency) of vancomycin hydrochloride according to the labeled amount, in 10 mL of the mobile phase, and use this solution as the test solution. Proceed as directed in the Purity (2) under Vancomycin Hydrochloride.

Water Not more than 5.0 % (0.1 g, volumetric titration, direct titration). Use a mixture of formamide for the Karl Fisher method and methanol for the Karl Fisher method (3 : 1).

Sterility Test It meets the requirement, when Vancomycin Hydrochloride is used in a sterile preparation.

Bacterial Endotoxins Less than 0.25 EU/mg (potency) of vancomycin, when Vancomycin Hydrochloride is used in a sterile preparation.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Injections It meets the requirement.

Uniformity of Dosage Units It meets the requirement.

Assay ***The Cylinder-plate method*** Perform the test as directed in the Assay under Vancomycin Hydrochloride. Weigh accurately the contents of not less than 10 containers of Vancomycin Hydrochloride for Injection. Weigh accurately an amount of the contents, equivalent to about 25 mg (potency) of Vancomycin Hydrochloride according to the labeled potency, and dissolve in water to make exactly 25 mL. Popet a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make solutions so that each mL contains 100 μg (potency) and 25 μg (potency), and use these solutions as the high concentration test solution and the low concentration test solution, respectively.

Containers and Storage ***Containers***—Hermetic containers.

Vasopressin Injection

Vasopressin Injection is an aqueous solution for injection. Vasopressin Injection contains synthetic vasopressin or the pressor principle, vasopressin, obtained from the posterior lobe of the pituitary of healthy cattles and pigs, from which the majority of the oxytocic principle, oxytocin, has been removed.
Vasopressin Injection contains not less than 85.0 % and not more than 120.0 % of the labeled vasopressin units.

Method of Preparation Prepare as directed under Injections, with vasopressin prepared by synthesis or obtained from the posterior lobe of the pituitary.

Description Vasopressin Injection is a clear and colorless liquid, is odorless or has a slight, characteristic odor.

pH—3.0 ~ 4.0.

Purity ***Oxytocic principle***—When tested by the following procedure, Vasopressin Injection contains not more than 0.6 oxytocin units for each determined 10 vasopressin units.

Standard stock solution—Dissolve 200 units of Oxytocin RS, according to the labeled units, in exactly 10 mL of diluted acetic acid (100) (1 in 400). Pipet 1 mL of this solution, and add diluted glacial acetic acid (1 in 400) to make exactly 10 mL. Store in a cold place, avoiding freezing. Use within 6 months from the date of preparation.

Standard solution—Dilute the standard stock solution with Isotonic Sodium Chloride Injection so that each mL of the solution contains 0.020 oxytocin units.

Test solution—Assume oxytocin units are equivalent to 6/100 of the determined vasopressin units. Dilute Vasopressin Injection with Isotonic Sodium Chloride Injection so that each mL of the resulting solution is expected to contain 0.020 oxytocin unit.

Apparatus—Use the apparatus for the uterus contraction test, equipped with a thermostatic bath. Maintain a temperature of the bath at 37 °C to 38 °C with a variation of not more than 0.1 °C during the course of the test. Use a 100-mL Magnus' chamber for suspend-

ing the uterus vertically.

Test animal—Use healthy, virgin and metestrus guinea pigs weighing between 175 g and 350 g. They have been bred under conditions where they have been completely isolated from the sight and smell of males since the time of weaning.

Procedure—Immerse the Magnus' chamber in the bath maintained at a constant temperature, add Locke-Ringer's solution to the chamber and introduce oxygen into the solution at a moderate rate. Sacrifice a guinea pig by means of a blow on the head, immediately remove the uterus from the body, suspend it in toe chamber and connect one horn of the uterus to the lever with a thread. If necessary, weigh the lever provided that the weight is not changed throughout the Assay. Start the Assay after 15 to 30 minutes when the uterus is completely relaxed. Administer the same quantities, 0.1 to 0.5 mL, of the standard solution and the test solution to the Magnus' chamber alternately twice with regular intervals of between 10 and 20 minutes to contract the uterus, finally administer the standard solution in a quantity which is 25 % larger than the preceding doses and measure the height of every contraction. The mean height of uterus contraction caused by the standard solution is equal to or higher than that caused by the test solution. The height of contraction caused by the increased dose of the standard solution is distinctly higher than those caused by the preceding doses of the standard solution.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 15 EU/unit of vasopressin.

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Iinjection It meets the requirement.

Determination of Volume of Injection in Container It meets the requirement.

Assay (1) ***Test animals***—Use healthy male rats weighing between 200 g and 300 g.

(2) ***Standard stock solution***—Dissolve 200 units of Oxytocin RS, according to the labeled units, in exactly 10 mL of diluted acetic acid (100) (1 in 400). Pipet 1 mL of this solution, and add diluted acetic acid (100) (1 in 400) to make exactly 10 mL. Store in a cold place, avoiding freezing. Use within 6 months from the date of preparation.

(3) ***Standard solution***—Dilute the standard stock solution with Isotonic Sodium Chloride Injection so that 0.2 mL of the obtained solution causes blood pressure increases of between 35 mmHg and 60 mmHg in test animals when injected according to (4) and designate this solution as the high-dose standard solution (S_H). Then dilute this solution with Isotonic Sodium Chloride Injection, 1.5 to 2.0 times by volume and designate as the low-dose standard solution (S_L).

(4) ***Test solution***—Dilute an accurately measured volume of Vasopressin Injection with Isotonic Sodium Chloride Injection so that the obtained solution contains the same concentration in units as the high-dose standard solution based on the labeled units and designate it as the high-dose test solution (T_H). Then dilute this solution with Isotonic Sodium Chloride Injection 1.5 to 2.0 times by volume and designate as the low-dose test solution (T_L). Make the concentration ratio of S_H to S_L equal to the ratio of T_H to T_L. When the sensitivity of an animal is changed, adjust the concentration of S_H and T_H before the next set of assay is started. However, keep the same ratio of S_H to S_L and T_H to T_L as in the primary set.

(5) ***Dose of injection***—Although 0.2 mL of each solution is usually injected, the dose of injection can be determined based from preliminary tests or experiences. Inject the same volume throughout a set of tests.

(6) ***Procedure***—Inject subcutaneously 0.7 mL of a solution of ethyl carbamate (1 in 4) per 100 g of body weight to anesthetize the test animals and cannulate the trachea. Under artificial respiration (about 60 strokes per minute), remove a part of the second cervical vertebra, cut off the spinal cord and destroy the brain through the foramen magnum. Insert cannula filled with Isotonic Sodium Chloride Injection into the femoral vein. Through this cannula, inject the solution prepared by dissolving 200 heparin units of heparin sodium in 0.1 mL of Isotonic Sodium Chloride Injection per 100 g of body wight and then immediately inject 0.3 mL of Isotonic Sodium Chloride Injection. Insert a cannula into a carotid artery and connect the cannula to a manometer for blood pressure measurement with vinyl tube. The cannula and the vinyl tube have previously been filled with Isotonic Sodium Chloride Injection. Inject the standard and the test solutions at regular intervals of 10 to 15 minutes into the femoral vein through the cannula followed by 0.3 mL of the Isotonic Sodium Chloride Injection when the blood pressure increases caused by each solution returns to the original level. Measure the height of blood pressure increases within 0.67 kPa on the kymogram. Maintain a constant temperature between 20 °C and 25 °C during the assay.

In advance, make four pairs from S_H, S_L, T_H, T_L as follows.

Randomize the order of injection for pairs, but keep the order of injection within pairs as indicated.

Pair 1: S_H, T_L, Pair 2: S_L, T_H,
Pair 3: T_H, S_L, Pair 4: T_L, S_H.

Carry out this Assay using the same animals throughout a set of four pairs of observations. Perform this assay with two sets. If necessary, however, use the different animals for both sets of tests.

(7) ***Calculation***—Subtract increases of blood pressure caused by the low dose from those caused by the high dose in the Pairs 1, 2, 3 and 4 of each set and obtain the responses y_1, y_2, y_3 and y_4, respectively. Sum up y_1, y_2, y_3 and y_4 to obtain Y_1, Y_2, Y_3 and Y_4, in the same way.

Units in 1 mL of Vasopressin Injection = antilog M × units in 1 mL of high-dose standard solution × $\frac{b}{a}$

$$M = \frac{IY_a}{Y_b}$$

$$I = \log\frac{S_H}{S_L} = \log\frac{T_H}{T_L}$$

$$Y_a = -Y_1 + Y_2 + Y_3 - Y_4$$

$$Y_b = Y_1 + Y_2 + Y_3 + Y_4$$

a: Volume (mL) of Vasopressin Injection sampled,
b: Total volume (mL) of the high-dose sample solution, prepared by diluting with Isotonic Sodium Chloride Injection.

Compute L (P = 0.95) by the following equation and confirm L to be 0.15 or less. If L exceeds 0.15, repeat the test, improving the conditions of the assay or increasing the number of sets until L reaches 0.15 or less.

$$L = 2\sqrt{(C-1)(CM^2 + I^2)}$$

$$C = \frac{Y_b^2}{Y_b^2 - 4fs^2t^2}$$

f: Number of sets

$$s^2 = \frac{\sum y^2 - \frac{Y}{f} - \frac{Y'}{4} + \frac{Y_b^2}{4f}}{n}$$

Σy_2: Sum of each squares of y_1, y_2, y_3 and y_4 of each group.

$$Y = Y_1^2 + Y_2^2 + Y_3^2 + Y_4^2$$

Y': Multiply the sum of y_1, y_2, y_3 and y_4 of one group and sum all of each group.

$$n = 3(f-1)$$

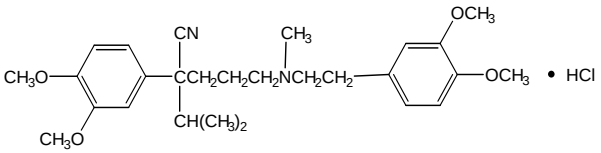
t^2: Value of the table of Assay for Insulin for Injection, to n of calculating s^2.

Containers and Storage ***Containers***—Hermetic containers.

Storage—In a cold place, and avoid freezing.

Expiration Date 36 months after preparation.

Verapamil Hydrochloride



Iproveratril Hydrochloride $C_{27}H_{38}N_2O_4$·HCl: 491.06

2-(3,4-Dimethoxyphenyl)-5-[2-(3,4-dimethoxyphenyl)ethyl-methylamino]-2-propan-2-ylpentanenitrile hydrochloride [*152-11-4*]

Verapamil Hydrochloride, when dried, contains not less than 98.5 % and not more than 101.0 % of verapamil hydrochloride ($C_{27}H_{38}N_2O_4$·HCl).

Description Verapamil Hydrochloride is a white, crystalline powder and is odorless.
Verapamil Hydrochloride is freely soluble in methanol, in acetic acid (100) or in chloroform, soluble in ethanol (95) or in acetic anhydride, sparingly soluble in water and practically insoluble in ether.

Identification (1) To 2 mL of a solution of Verapamil Hydrochloride (1 in 50), add 5 drops of Reinecke salt TS: a pale red precipitate is produced.

(2) Determine the absorption spectra of solutions of Verapamil Hydrochloride and Verapamil Hydrochloride RS in 0.01 mol/L hydrochloric acid TS (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Determine the infrared spectra of Verapamil Hydrochloride and Verapamil Hydrochloride RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(4) A solution of Verapamil Hydrochloride (1 in 50) responds to the Qualitative Tests for chloride.

Melting Point 141 ~ 145 °C.

pH Dissolve 1.0 g of Verapamil Hydrochloride in 20 mL of freshly boiled and cooled water by warming and cool: the pH of this solution is between 4.5 and 6.5.

Purity (1) ***Clarity and color of solution***— Dissolve 1.0 g of Verapamil Hydrochloride in 20 mL of water by warming: the solution is clear and colorless

(2) ***Heavy metals***—Proceed with 1.0 g of Verapamil Hydrochloride according to Method 2 and perform the test. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Arsenic***—Prepare the test solution with 1.0 g of Verapamil Hydrochloride according to Method 3 and perform the test (not more than 2 ppm).

(4) ***Related substances***—Dissolve 0.50 g of Verapamil Hydrochloride in 10 mL of chloroform and use this solution as the test solution. Pipet 1.0 mL of the test solution, add chloroform to make exactly 100 mL and use this solution as the standard stock solution. Pipet 5.0 mL of the standard stock solution, add chloroform to make exactly 100 mL and use this solution as the standard solution (1). Separately, pipet 5.0 mL of the standard stock solution, add chloroform to make exactly 50 mL and use this solution as the standard solution (2). Perform the test with the test solution and the standard solutions (1) and (2) as directed under Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solutions (1) and (2) on two plates of silica gel for thin-layer chromatography. With the one plate, develop the plate with a mixture of cyclohexane and dimethylamine (17 : 3) to a distance of about 15 cm, air-dry the plate, heat at 110 °C for 1 hour and cool. Examine immediately after spraying evenly iron (III) chloride-iodine TS on the plate: the three spots, having more intense color in the spots than the principal spot and the original point from the test solution, are not more intense than the spot from the standard solution (1) in color. With another plate, develop the plate with a mixture of toluene, methanol, acetone and acetic acid (100) (14 : 4 : 1 : 1) and perform the test in the same manner.

Loss on Drying Not more than 1.0 % (1 g, 105 °C, 2 hours).

Residue on Ignition Not more than 0.1 % (1 g).

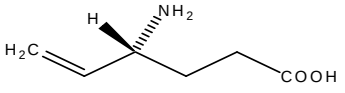
Assay Weigh accurately about 0.7 g of Verapamil Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7 : 3) and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 49.11 mg of $C_{27}H_{38}N_2O_4 \cdot HCl$

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Vigabatrin



and enantiomer

$C_6H_{11}NO_2$: 129.16

4-Aminohex-5-enoic acid [*68506-86-5*]

Vigabatrin contains not less than 98.0 % and not more than 102.0 % of vigabatrin ($C_6H_{11}NO_2$), calculated on an anhydrous basis.

Description Vigabatrin appears as white powder. Vigabatrin is very soluble in water.

Identification (1) Determine the infrared spectra of Vigabatrin and Vigabatrin RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) In the Assay, the retention time of the principal peak from the test solution is the same as that from the standard solution.

Specific Optical Rotation $[\alpha]_D^{20}$: +0.5 ~ –0.5° (2 g, water, 10 mL, 100 mm).

Purity (1) ***Heavy metals*** —Proceed the test with 2.0 g of Vigabatrin according to Method 2. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Ethanol***—Dissolve 2 g of Vigabatrin, accurately weighed, in 10 mL of a solution containing 0.0025 % of 1,2-dichloroethane (internal standard) in water in a 20 mL-sized headspace vial. Heat the vial at 60 °C for 30 minutes, and use the solution as the test solution. Separately, prepare a solution containing 0.025 % of dehydrated ethanol and 0.0025 % of 1,2-dichloroethane in water and use this solution as the standard solution. Use water as the blank. Perform the test with the blank solution, the test solution and the standard solution as directed under the head-space Gas Chromatography according to the following conditions. Using the chromatograms obtained with the test solution and the standard solution, determine the ratios of the peak area of ethanol to that of the internal standard for the test solution and the standard solution, and calculate the content of ethanol in the portion of Vigabatrin: not more than 0.6 % of ethanol is found.

Operating conditions

Detector: A flame ionization detector.

Column: A fused-silica column 0.32 mm internal diameter and 60 m in length, coated with a 1.0 μm film of bonded methylsilicone for gas chromatography.

Carrier gas: Helium

Injector temperature: 150 °C.

Detector temperature: 250 °C.

Column temperature: Maintain an initial temperature at 35 °C for 12 minutes, and increase to 175 °C at a constant rate of 10 °C per minute.

(3) ***Related substances***—The sum of the related substances determined by methods (I) and (II) described below is not more than 0.5 %.

(I) Dissolve 0.4 g of Vigabatrin in the mobile phase to make 100 mL, and use this solution as the test solution. Separately, dissolve 4.0 mg of 3-aminopent-4-ene-1,1-dicarboxylic acid RS in the 100 mL of the mobile phase. Dilute 5.0 mL of this solution with the mobile phase to exactly 50 mL, and use this solution as the standard solution (1). Dissolve 4.0 mg of 5-vinyl-2-pyrrolidone RS in the mobile phase to make exactly 100 mL. Dilute 5.0 mL of this solution with the mobile phase to 50 mL, and use this solution as the standard solution (2). Dissolve 4.0 mg of (*E*)-4-amino-2-ethylidenebutyric acid hydrochloride RS in the mobile phase to make exactly 100 mL. Dilute 5.0 mL of this solution with the mobile phase to 50 mL, and use this solution as the standard solution (3). Dissolve 2.0 mg of 5-vinyl-2-pyrrolidone RS and 0.40 g of Vigabatrin RS in the mobile phase to make exactly 100 mL, and use this solution as the standard solution (4). Perform the test with 20 μL each of the test solution, the standard solutions (1), (2), and (3) as directed under Liquid Chromatography according to the following conditions. Determine the area of each peak by the automatic integration method. The areas of any peaks corresponding to 5-vinyl-2-pyrrolidone and *(E)*-4-amino-2-ethylidenebutyric acid obtained from the test solution are not greater than the areas of the peaks from the standard solutions (2) and (3), respectively (not more than 0.1 % of each); the area of any other secondary peak is not greater than the area of the peak from the standard solution (1) (not more than 0.1 %). Calculate the percentage content of 5-vinyl-2-pyrrolidone and *(E)*-4-amino-2-ethylidenebutyric acid using the areas of the peaks from the standard solutions (2) and (3) respectively and of any other related substance from the peak from the standard solution (1) taking, for the purposes of calculation, that this peak is equivalent to 0.1 %, and hence determine the sum of the contents of total related substances.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: Two stainless steel columns connected to each other in series.

Column 1: A stainless steel column about 4.6 mm internal diameter and 25 cm in length, packed with silica of which surface is modified with chemically-bonded hexylsilyl groups (5 μm in particle diameter).

Column 2: A stainless steel column about 4.6 mm internal diameter and 25 cm in length, packed with cation exchange resin (10 μm in particle diameter).

Mobile phase: A mixture of water, acetonitrile, and phosphate buffer solution (950 : 25 : 25).

Flow rate: 1.0 mL/min.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution (4) under the above operating conditions, 5-vinyl-2-pyrrolidone and vigabatrin are eluted in this order with the resolution between the peaks of 5-vinyl-2-pyrrolidone and vigabatrin being not less than 1.5.

Time span of measurement: 2 times as long as the retention time of vigabatrin.

Phosphate buffer solution: Dissolve 58.5 g of sodium dihydrogen phosphate in water, add 23 mL of phosphoric acid, and dilute with water to 1000 mL.

(II) Dissolve 20.0 mg of Vigabatrin in 10 mL of water to make exactly 10 mL. To 1 mL of this solution, add 2 mL of a solution prepared by dissolving 7.7 g of boric acid in water, adjusting to pH 7.7 with 50 % sodium hydroxide solution and diluting to 250 mL with water. Add 3 mLof a solution of 0.16 % (9-fluorenyl)methyl chloroformate in acetone, mix and allow to stand for 5 minutes. Add 3 mL of ethyl acetate, shake vigorously for a few seconds, and allow to separate. Use the lower layer as the test solution and proceed the test within 8 hours of preparation. Separately, dissolve 2.0 mg of 4-aminobutyric acid and 0.2 g of Vigabatrin in water to make 100 mL. Using 1 mL of this solution, prepare the standard solution in the same manner. Perform the test with 25 μL of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine the peak area of 4-aminobutyric acid by the automatic integration method: the content of 4-aminobutyric acid in the portion of Vigabatrin is not more than 0.2 %.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 263 nm).

Column: A stainless steel column about 4.6 mm internal diameter and 25 cm in length, packed with 5 μm silica gel particles of which surface of which is modified by chemically-bonded phenyl groups.

Mobile phase: A mixture of sodium acetate buffer solution and acetonitrile (75 : 25).

Flow rate: 1.0 mL/min.

System suitability

System performance: When the procedure is run with 25 μL of the standard solution under the above operating conditions, (9-fluorenyl)methanol, 4-amino butyric acid, and vigabatrin are eluted in this order and their corresponding retention times are about 6 minutes, 9 minutes, and 14 minutes, respectively, with the resolution between the peaks of the derivatives of 4-aminobutyric acid and (9-fluoreny)methanol being not less 2.

Sodium acetate buffer solution: Dissolve 8.2 g of anhydrous sodium acetate in water, adjust the pH to 4.2 with acetic acid (100) and dilute to 2000 mL with water.

Water Not more than 0.5 % (0.3 g, purified methanol, 50 mL, volumetric titration, direct titration).

Assay Dissolve 0.2 g each of Vigabatrin and Viga-

batrin RS, accurately weighed, in water to make 100 mL. Use these solutions as the test solution and the standard solution, respectively. Perform the test with 20 μL each of these solutions as directed in the Liquid Chromatography according to the following conditions. Calculate the peak areas, A_T and A_S, of vigabatrin of these solutions.

$$\text{Amount (mg) of vigabatrin } (C_6H_{11}NO_2) = \text{Amount (mg) of Vigabatrin RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column about 4.6 mm internal diameter and 25 cm in length, packed with 10 μm cation exchange resin.

Mobile phase: A mixture of phosphate buffer solution, methanol, and acetonitrile (1000:40:4).

Flow rate: 1.5 mL/min.

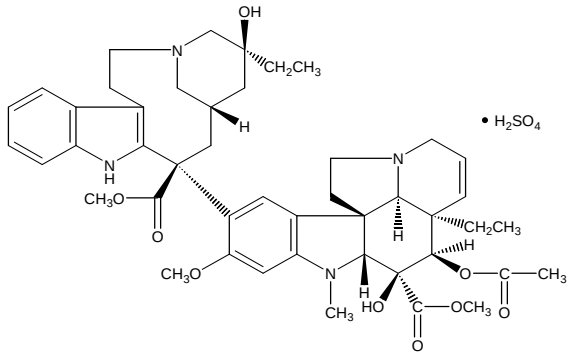
System suitability

System performance: Dissolve 2.0 mg of 5-vinyl-2-pyrrolidone RS and 0.2 g of Vigabatrin RS in water to make 100 mL. When the procedure is run with 20 μL of this solution, 5-vinyl-2-pyrrolidone and vigabatrin are eluted in this order and the resolution between the peaks of 5-vinyl-2-pyrrolidone and vigabatrin being not less than 1.5.

Phosphate buffer solution—Dissolve 3.4 g of potassium dihydrogen phosphate in water, adjust the solution pH to 2.8 by phosphoric acid, and dilute with water to 1000 mL.

Containers and Storage ***Containers***—Well-closed containers.

Vinblatine Sulfate



$C_{46}H_{58}N_4O_9 \cdot H_2SO_4$: 909.05

Methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-acetyloxy-3a-ethyl-9-[(5*S*,7*S*,9*S*)-5-ethyl-5-hydroxy-9-methoxycarbonyl-1,4,5,6,7,8,9,10-octahydro-3,7-methano-3-azacycloundecino[5,4-*b*]indol-9-yl]-5-hydroxy-8-methoxy-6-methyl-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate monosulfate [*143-67-9*]

Vinblastine Sulfate contains not less than 96.0 % and not more than 102.0 % of vinblastine sulfate ($C_{46}H_{58}N_4O_9 \cdot H_2SO_4$), calculated on the dried basis.

Description Vinblastine Sulfate is a white to pale yellow powder.
Vinblastine Sulfate is soluble in water, sparingly soluble in methanol and practically insoluble in ethanol (95) or ether.
Vinblastine Sulfate is hygroscopic.

Identification (1) Determine the absorption spectra of the solutions of Vinblastine Sulfate and Vinblastine Sulfate RS (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectra of Vinblastine Sulfate and Vinblastine Sulfate RS as directed in the potassium bromide disk method under Infrared Spectrophotometery: both spectra exhibit similar intensities of absorption at the same wavenumbers..

(3) A solution of Vinblastine Sulfate (1 in 100) responds to the Qualitative Tests for sulfate.

Specific Optical Rotation $[\alpha]_D^{20}$: -28 ~ -35 ° (20 mg, calculated on the dried basis, methanol, 10 mL, 100 mm).

pH Dissolve 15 mg of Vinblastine Sulfate in 10 mL of water: the pH of this solution is between 3.5 and 5.0.

Purity (1) ***Clarity and color of solution***—Dissolve 10 mg of Vinblastine Sulfate in 10 mL of water: the solution is clear and colorless.

(2) ***Related substances***—Dissolve about 4 mg of Vinblastine Sulfate in 10 mL of water and use this solution as the test solution. Pipet 1.0 mL of the test solution, add water to make exactly 25 mL and use this solution as the standard solution. Perform the test with 200 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the area of any peak other than the main peak is not larger than 1/4 of the peak area of vinblastine from the standard solution, and the total area of peaks other than the main peak is not larger than 3/4 of the peak area of vinblastine from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: To exactly 2.5 mL of the standard solution, add water to make exactly 100 mL. Confirm that the peak area of vinblastine obtained from 200 μL of this solution is equivalent to 1.7 to 3.3 % of that from the standard solution.

System repeatability: When the test is repeated 6 times with 200 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of vinblastine is not more than 1.5 %.

Time span of measurement: About 4 times as long as the retention time of vinbalstine beginning after the solvent peak.

Loss on Drying Perform the test with about 10 mg of Vinblastine Sulfate as directed in Method 2 under the Thermal Analysis according to the following conditions: not more than 15.0 %.

Operating conditions

Heating rate: 5 °C/minute
Temperature range: room temperature to 200 °C
Atmospheric gas: dried nitrogen
Flow rate of atmospheric gas: 40 mL/minute

Sterility Test It meets the requirement, when Vinblastine Sulfate is used in a sterile preparation.

Bacterial Endotoxins Less than 10.0 EU/mg of vinblastine sulfate, when Vinblastine Sulfate is used in a sterile preparation.

Assay Weigh accurately about 10 mg of Vinblastine Sulfate and Vinblastine Sulfate RS (previously determine the loss on drying in the same manner as Vinblastine Sulfate), dissolve in water to make exactly 25 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with exactly 20 μL each of these solutions as directed under the Liguid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of vinbalstine.

$$\text{Amount (mg) of vinblastine sulfate } (C_{46}H_{58}N_4O_9 \cdot H_2SO_4) = \text{Amount (mg) of Vinblastine Sulfate RS, calculated on the dried basis} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 262 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Mobile phase: To 7 mL of diethylamine, add water to make 500 mL and adjust the pH 7.5 with phosphoric acid. To 380 mL of this solution, add 620 mL of a mixture of methanol and acetonitrile (4 : 1).

Flow rate: Adjust the flow rate so that the retention time of vinblastine is about 8 minutes.

System suitability

System performance: Dissolve 10 mg each of Vinblastine Sulfate and vincristine sulfate in 25 mL of water. When the procedure is run with 20 μL of this solution under the above operating conditions, vincristine and vinblastine are eluted in this order with a resolution between their peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of vinblastine is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and not exceeding –20 °C.

Vinblatine Sulfate for Injection

Vinblastine Sulfate for Injection is a preparation for injection which is reconstituted before use. When dried, Vinblastine Sulfate for Injection contains not less than 90.0 % and not more than 110.0 % of the labeled amount of vinblastine sulfate ($C_{46}H_{58}N_4O_9 \cdot H_2SO_4$: 909.05).

Method of Preparation Prepare as directed under Injections, with Vinblastine Sulfate.

Description Vinblastine Sulfate for Injection is a white to pale yellow, light mass or powder.
Vinblastine Sulfate for Injection is freely soluble in water.
The pH of its aqueous solution (1 in 1000) is 3.5 to 5.0.

Identification Proceed as directed in the Identification (1) under Vinblastine Sulfate.

Purity ***Related substances***—Dissolve 4 mg of Vinblastine Sulfate for Injection in 10 mL of water and use this solution as the test solution. Pipet 1.0 mL of the test solution, add water to make exactly 25 mL and use this solution as the standard solution. Perform the test with 200 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the area of any peak other than the main peak of the test solution is not larger than 1/2 of the peak area of vinblastine from the standard solution, and the total area of any peaks other than the main peak from the test solution is not larger than 2 times the peak area of vinblastine from the standard solution.

Operating conditions

Perform as directed in the operating conditions in the Purity (2) under Vinblastine Sulfate.

System suitability

Perform as directed in the system suitability in the Purity (2) under Vinblastine Sulfate.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 10 EU/mg

Foreign Insoluble Matter Test It meets the requirement.

Insoluble Particulate Matter Test for Iinjection It meets the requirement.

Uniformity of Dosage Units It meets the requirement when the content uniformity test is performed according to the following method.

Dissolve 1 Vinblastine Sulfate Injection in wate to make exactly *V* mL so that each mL contains abut 0.4 mg of vinblastine sulfate ($C_{46}H_{58}N_4O_9 \cdot H_2SO_4$) according to the labeled amount, and use this solution as the test solution. Separately, weigh accurately about 10 mg of Vinblastine Sulfate RS (previously determine the loss on drying in the same manner as Vinblastine Sulfate), dissole in water to make exactly 25 mL, and use this solution as the standard solution. Proceed as directed in the Asay under Vinblastine Sulfate.

$$\text{Amount (mg) of vinblastine sulfate } (C_{46}H_{58}N_4O_9 \cdot H_2SO_4) = \text{Amount of Vinblastine Sulfate RS, calculated on the dried basis} \times \frac{A_T}{A_S} \times \frac{25}{V}$$

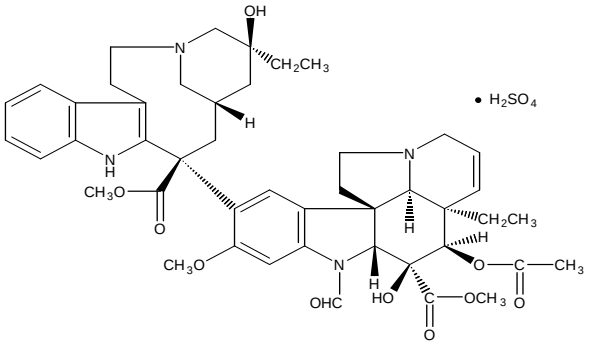
Assay Take an amount of Vinblastine Sulfate for Injection, equivalent to 0.10 g of vinblastine sulfate ($C_{46}H_{58}N_4O_9 \cdot H_2SO_4$), dissolve each content with a suitable amount of water, transfer into a 100-mL volumetric flask, wash each container with water, transfer the washings into the volumetric flask, and add water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 25 mL, and use this solution as the test solution. Separately, weigh accurately about 10 mg of Vinblastine Sulfate RS (previously determine the loss on drying in the same manner as Vinblastine Sulfate), dissolve in water to make exactly 25 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Vinblastine Sulfate.

$$\text{Amount (mg) of vinblastine sulfate } (C_{46}H_{58}N_4O_9 \cdot H_2SO_4) = \text{Amount (mg) of Vinblastine Sulfate RS, calculated on the dried basis} \times \frac{A_T}{A_S} \times 10$$

Containers and Storage ***Containers***—Hermetic cont-ainers.

Storage—Light-resistant, and in a cold place.

Vincristine Sulfate



$C_{46}H_{56}N_4O_{10} \cdot H_2SO_4$: 923.04

Methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-acetyloxy-3a-ethyl-9-[(5*S*,7*S*,9*S*)-5-ethyl-5-hydroxy-9-methoxycarbonyl-1,4,5,6,7,8,9,10-octahydro-3,7-methano-3-azacycloundecino[5,4-*b*]indol-9-yl]-6-formyl-5-hydroxy-8-methoxy-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate monosulfate [*2068-78-2*]

Vincristine Sulfate contains not less than 95.0 % and not more than 105.0 % of vincristine sulfate ($C_{46}H_{56}N_4O_{10} \cdot H_2SO_4$), calculated on the dried basis.

Description Vincristine Sulfate is a white to pale yellowish white powder.

Vincristine Sulfate is very soluble in water and practically insoluble in ethanol (95).

Vincristine Sulfate is hygroscopic.

Specific Optical Rotation—$[\alpha]_D^{20}$: +28.5 ~ +35.5° (0.20 g, calculated on the dried basis, water, 10 mL, 100 mm).

Identification (1) Determine the absorption spectra of solutions of Vincristine Sulfate and Vincristine Sulfate RS (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of abosprtion at the same wavelengths.

(2) Determine the infrared spectra of Vincristine Sulfate and Vincristine Sulfate RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Vincristine Sulfate (1 in 100) responds to the Qualitative Tests for sulfate.

pH Dissolve 10 mg of Vincristine Sulfate in 10 mL of water: the pH of this solution is between 3.5 and 4.5.

Purity (1) ***Clarity and color of solution***—Dissolve

50 mg of Vincristine Sulfate in 10 mL of water: the solution is clear and colorless.

(2) ***Related substances***—Dissolve 10 mg of Vincristine Sulfate in 10 mL of water, and use this solution as the test solution. Pipet 2 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 200 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the peak area of desacetyl vincristine and vinblastine, having the relative retention times of about 0.9 and about 1.6 with respect to vincristine, respectively, obtained from the test solution are not larger than 1/8 times and 3/20 times, respectively, the peak area of vincristine from the standard solution, and the area of the peak other than vincristine, desacetyl vincristine and vinblastine from the test solution are not larger than 1/4 times the peak area of vincristine from the standard solution. Furthermore, the total area of the peaks other than vincristine from the test solution is not larger than the peak area of vincristine from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 297 nm).

Column: A stainless steel column, about 4.6 mm in internal diameter and about 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C

Mobile phase A: Methanol

Mobile phase B: A mixture of water and diethylamine (197 : 3), adjusted to pH 7.5 with phosphoric acid.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time (min)	Mobile phase A (vol %)	Mobile phase B (vol %)
0-12	62	38
12-27	62→92	38→8

Flow rate: Adjust the flow rate so that the retention time of vincristine is about 15 minutes.

System suitability

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 200 mL. Confirm that the peak area of vincristine obtained from 200 μL of this solution is equivalent to 1.75 to 3.25 % of that from 200 μL of the standard solution.

System performance: Dissolve 15 mg each of Vincristine Sulfate and vinblastine sulfate in 100 mL of water. When the procedure is run with 200 μL of this solution under the above operating conditions, vincristine and vinblastine are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 200 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of vincristine is not more than 1.5 %.

Time span of measurement: About 1.7 times as long as the retention time of vincristine, beginning after the solvent peak.

Loss on Drying Perform the test with about 10 mg of Vincristine Sulfate as directed in Method 2 under Thermal Analysis according to the following conditions: not more than 12.0 %.

Operating conditions

Heating rate: 5 °C/minute

Temperature range: Room temperature to 200 °C

Atmospheric gas: Dried nitrogen

Flow rate of atmospheric gas: 40 mL/minute

Assay Weigh accurately about 10 mg each of Vincristine Sulfate and Vincristine Sulfate RS (separately determine the loss on drying in the same conditions as Vincristine Sulfate), dissolve each in water to make exactly 10 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with exactly 10 μL each of these solutions as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of vincristine.

$$\text{Amount (mg) of vincristine sulfate } (C_{46}H_{56}N_4O_{10}\cdot H_2SO_4) = \text{Amount (mg) of Vincristine Sulfate RS, calculated on the dried basis} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 297 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: Adjust the pH to 7.5 of a mixture of water and diethylamine (59 : 1) with phosphoric acid. To 300 mL of this solution add 700 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of vinblastine is about 7 minutes.

System suitability

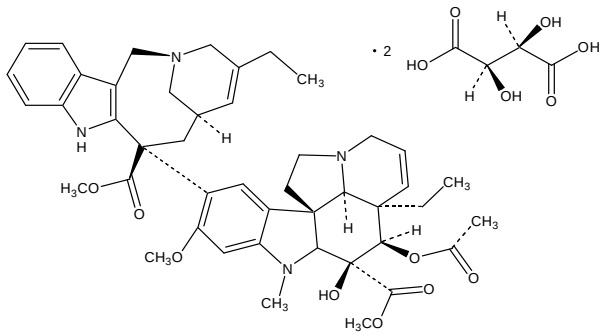
System performance: Dissolve 5 mg each of Vincristine Sulfate and vinblastine sulfate in 5 mL of water. When the procedure is run with 10 μL of this solution under the above operating conditions, vincristine and vinblastine are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of vincristine is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and not exceeding –20 °C.

Vinorelbine Tartrate



$C_{45}H_{54}N_4O_8 \cdot 2C_4H_6O_6$: 1079.11

Methyl (1*R*,9*R*,10*R*,11*R*,12*R*,19*R*)-11-(acetyloxy)-12-ethyl-4-[(12*S*,14*R*)-16-ethyl-12-(methoxycarbonyl)-1,10-diazatetracyclo[12.3.1.03,11.04,9]octadeca-3(11),4,6,8,15-pentaen-12-yl]-10-hydroxy-5-methoxy-8-methyl-8,16-diazapentacyclo[10.6.1.0^{1,9}.02,7.016,19]nonadeca-2,4,6,13-tetraene-10-carboxylate *bis*[(2*R*,3*R*)-2,3-dihydroxybutanedioate] [*125317-39-7*]

Vinorelbine Tartrate contains not less than 98.0 % and not more than 102.0 % of vinorelbine tartrate ($C_{45}H_{54}N_4O_8 \cdot 2C_4H_6O_6$), calculated on the anhydrous basis.

Description Vinorelbine Tartrate is a white to yellow or bright brown amorphous powder.
Vinorelbine Tartrate is freely soluble in water.

Identification (1) Dissolve 50 mg of Vinorelbine Tartrate in 1 mL of water. To 0.1 mL of this solution, add 0.1 mL of 10 % potassium bromide solution, 0.1 mL of 2 % resorcinol solution, and 3 mL of sulfuric acid. Heat on a hot water bath for 5 to 10 minutes until a deep blue color develops. Allow to cool, and pour the solution into water. The color changes to red.

(2) Dissolve 10 mg each of Vinorelbine Tartrate and Vinorelbine Tartrate RS in 5 mL of water, add 0.5 mL of 5 mol/L sodium hydroxide, and extract with 5 mL of methylene chloride. Filter the organic extracts through anhydrous sodium sulfate, and evaporate the organic extracts to about 0.5 mL. Use these solutions as the test solution and the standard solution. Determine the infrared spectra of these solutions as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) The retention time of the major peak obtained from the test solution corresponds to that from the standard solution (2), as obtained in the test for related substances in the Purity.

pH The pH of a solution obtained by dissolving 0.5 g of Vinorelbine Tartrate in 50 mL of water is between 3.3 and 3.8.

Purity (1) ***Clarity and color of solution*—**Dissolve 0.1 g of Vinorelbine Tartrate in 10 mL of water: the solution is clear. Determine the absorbance of this solution as directed under Ultraviolet-visible Spectrophotometery: its absorption, determined at 420 nm using water as the blank, is not more than 0.03.

(2) ***Related substances*—**Dissolve 35 mg of Vinorelbine Tartrate, accurately weighed, in the mobile phase to make exactly 25 mL, and use this solution as the test solution. Separately, dissolve 35 mg of Vinorelbine Tartrate, accurately weighed, in the mobile phase to make exactly 25 mL, and use this solution as the standard solution (1). Pipet 1.0 mL of the standard solution (1), and dilute with the mobile phase to 50 mL. Pipet 1 mL of this solution, dilute with the mobile phase again to 100 mL, and use this solution as the standard solution (2). Perform the test with 20 μL each of the test solution and the standard solution (2) as directed under Liquid Chromatography, determine their major peak areas by the automatic integration method, and calculate the percentage of each related substance in the portion of Vinorelbine Tartrate. The photodegradation product is not more than 0.3 %, any individual related substance or coeluted related substances comprising an individual peak is not more than 0.2 %, and total impurities, excluding the photodegradation product, is not more than 0.7 %. Disregard any peak areas less than or equal to one-half of the peak area obtained for vinorelbine from the standard solution (2).

$$\text{Amount (\%) of each related substance} = 100 \times \frac{A_i}{A_S}$$

A_i: Peak area of each related substance obtained from the test solution.

A_S: Sum of all the peak areas obtained from the test solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 267 nm).

Column: A stainless steel column about 3.9 mm internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Flow rate: 1.0 mL/min.

Mobile phase: Dissolve 1.22 g of sodium 1-decanesulfonate in 620 mL of methanol. Add 380 mL of phosphate buffer.

System suitability

System performance: Dissolve accurately weighed quantities of Vinorelbine Tartrate RS and Vinorelbine Related Substance I RS in water to obtain a solution having known concentrations of about 1.4 mg per mL and 0.01 mg per mL, respectively. Expose a portion of this solution in a suitable xenon lamp apparatus capable of supplying a dose of 1600 KJ/m^2 between 310 and 800 nm at a power of 500 W/m^2 for about one hour, in order to generate an additional photodegradation product (3',4',7,8-tetradehydro-3,4'-dideoxy-3,6-epoxy-6,7-dihydro-C'-norvincaleukoblastine) having a relative retention time of about 0.8. When the procedure is run with 20 μL of this solution under the above operating conditions, the retention time for vinorelbine is about 13.5 minutes, the relative retention times are about 0.8 for the photodegradation product and 1.2 for vinorelbine related substance I, and the resolution between the peaks of vinorelbine tartrate and vinorelbine related substance I is not less than 1.1.

Time span of measurement: About 3 times as long as the retention time of vinorelbine.

Phosphate buffer—Dissolve 6.9 g of monobasic sodium phosphate in 900 mL of water. Adjust with phosphoric acid to a pH of 4.2, and dilute with water to 1000 mL.

Water Not more than 4.0 % (1 g, volumetric titration, direct titration)

Residue on Ignition Not more than 0.1 % (1 g)

Assay Dissolve about 0.35 g of Vinorelbine Tartrate, accurately weighed, in 40 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 53.96 mg of $C_{45}H_{54}N_4O_8 \cdot 2C_4H_6O_6$.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and in a freezer.

Vitamin A Oil

Retinol Oil

Vitamin A Oil is synthetic vitamin A esters diluted with fixed oils.

Vitamin A Oil contains not less than 30000 vitamin A units per g.

Vitamin A Oil may contain suitable antioxidants.

Vitamin A Oil contains not less than 90.0 % and not more that 120.0 % of the labeled units of vitamin A.

Description Vitamin A Oil is yellow to yellow-brown clear or slightly turbid oil, is odorless or has a faint, characteristic odor.

Decomposition of Vitamin A Oil is accelerated upon exposure to air or light.

Identification Dissolve Vitamin A Oil, Retinol Acetate RS, and Retinol Palmitate RS, equivalent to 15000 units, in 5 mL of petroleum ether, and use these solutions as the test solution, standard solution (1), and standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 5 μL each of the test solution, standard solution (1), and standard solution (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and ether (12 : 1) to a distance of about 10 cm, and air-dry the plate. Spray evenly antimony (III) chloride TS on the plate: the principal spot obtained from the test solution has the same color and R_f value as the blue principal spot obtained from standard solution (1) or standard solution (2).

Purity (1) ***Acid***—Dissolve 1.2 g of Vitamin A Oil in 30 mL of a mixture of neutralized ethanol and ether (1 : 1), boil gently for 10 minutes under a reflux condenser, cool and add 5 drops of phenolphthalein TS and 0.60 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(2) ***Rancidity***—No unpleasant odor of rancid oil is perceptible by warming Vitamin A Oil.

(3) ***Related substances***—Vitamin A Oil meets the conditions determined as directed in Method 1 under the Vitamin A Assay, or its *f* value determined as directed in Method 2 under the Vitamin A Assay is not less than 0.85.

Assay Proceed as directed under the Vitamin A Assay.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant, and almost well-filled or under nitrogen atmosphere.

Vitamin A Oil Capsules

Vitamin A Capsules

Vitamin A Oil Capsules contain not less than 90.0 % and not more than 130.0 % of the labeled units of Vitamin A.

Method of Preparation Prepare as directed under Capsules, with Vitamin A Oil.

Description The content of Vitamin A Oil Capsules conforms to the requirement of Description under Vitamin A Oil.

Identification Proceed the test with the content of Vitamin A Oil Capsules as directed in the Identification under Vitamin A Oil.

Disintegration Test It meets the requirement.

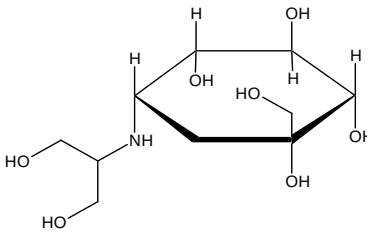
Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately 20 Vitamin A Oil Capsules, cut open, transfer the oil contents, mix well and proceed with the oil as directed under Vitamin A Assay. Wash the capsules with a small amount of ether, allow to stand at room temperature to evaporate the ether and weigh accurately. Calculate the weight of Vitamin A Oil from the difference between the weights before and after the above-described procedure. Calculate the Vitamin A units per 1 capsule from the weight and the Vitamin A units of the oil.

Containers and Storage ***Containers***—Well-closed containers.

Storage—Light-resistant.

Voglibose



$C_{10}H_{21}NO_7$: 267.28

(1*S*,2*S*,3*R*,4*S*,5*S*)-5-(1,3-dihydroxypropan-2-ylamino)-1-(hydroxymethyl)cyclohexane-1,2,3,4-tetrol
[*83480-29-9*]

Voglibose contains not less than 99.5 % and not more than 101.0 % of voglibose ($C_{10}H_{21}NO_7$), calculated on the anhydrous basis.

Description Voglibose appears as white crystals or crystalline powder.

Voglibose is freely soluble in water, soluble in acetic acid (100), slightly soluble in methanol, and very slightly soluble in ethanol (99.5).

Voglibose dissolves in 0.1 mol/L hydrochloric acid TS.

Identification (1) Determine the infrared spectra of Voglibose and Voglibose RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Determine the ^1H spectrum of a solution of Voglibose in heavy water for nuclear magnetic resonance spectroscopy (3 in 70) as directed under Nuclear Magnetic Resonance Spetroscopy, using sodium 3-trimethylsilyl-propionate-d_4 for nuclear magnetic resonance spetroscopy as an internal standard compound: it exhibits 2 double signals A at about δ 1.5 ppm, 2 double signals B at around δ 2.1 ppm, and a multiple signal C at about δ 2.9 ppm, and a multiple signal D between δ 3.4 ppm and δ 3.9 ppm. The area intensity ratio of each signal, A : B : C : D, is about 1 : 1 : 1 : 10.

Specific Optical Rotation $[\alpha]_D^{20}$: +45 ~ +48° (0.2g calculated on the anhydrous basis, 0.1 mol/L hydrochloric acid TS, 20 mL, 100 mm).

Melting Point 163 ~ 168 °C.

pH Dissolve 1.0 g of Voglibose in 10 mL of water: the pH of the solution is between 9.8 and 10.4.

Purity (1) ***Heavy metals***—Proceed with 1.0 g of Voglibose according to Method 1, and perform the test. Adjust the pH of the test solution between 3.0 and 3.5 with dilute hydrochloric acid instead of dilute acetic acid. Prepare the control solution with 1.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Related substances***—Dissolve 50 mg of Voglibose in 50 mL of the mobile phase, and use this solution as the test solution. Pipet 1.0 mL of the test solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following operating conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than voglibose is not larger than 0.2 times the peak area of voglibose from the standard solution. For the calculation of the total area, use the area of the peaks, having the relative retention time of about 1.7, about 2.0 and about 2.3 to voglibose, after multiplying by their relative response factors, 2, 2 and 2.5, respectively.

Opertating conditions

Apparatus: Use a pumping system for the mobile phase, pumping system for the reaction reagent, sample injection port, column, reaction coil, cooling coil, detector and recording device. Attach a 3-way tube to the outlet for the mobile phase of the column, connect the pumping system for the reaction reagent and the reaction coil, and join outlet of the reaction coil to the spectrofluorometer.

Detector: Spectrofluorometer (excitation wavelength: 350 nm, fluorescence wavelength: 430 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 25 cm in length, packed

with pentaethylenehexaaminated polyvinyl alcohol polymer bead for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C.

Reaction coil: A polytetrafluoroethylene tube about 0.5 mm in internal diameter and about 20 m in length.

Cooling coil: A polytetrafluoroethylene tube about 0.3 mm in internal diameter and about 2 m in length.

Mobile phase: To 1.56 g of sodium dihydrogen phosphate hydrate add water to make 500 mL. To this solution add a solution, prepared by dissolving 3.58 g of disodium hydrogen phosphate dodecahydrate in water to make 500 mL, to adjust to pH 6.5. To 370 mL of this solution add 630 mL of acetonitrile.

Reaction reagent: Dissolve 6.25 g of taurine and 2.56 g of sodium periodate in water to make 1000 mL.

Reaction temperature: A constant temperature of about 100 °C

Cooling temperature: A constant temperature of about 15 °C

Flow rate of mobile phase: Adjust the flow rate so that the retention time of voglibose is about 20 minutes.

Flow rate of reaction reagent: Same as the flow rate of the mobile phase

System suitability

Test for required detectability: Pipet 10 mL of the standard solution, and add the mobile phase to make exactly 100 mL. Confirm that the peak area of voglibose obtained from 50 μL of this solution is equivalent to 7 to 13 % of that of voglibose obtained from 50 μL of the standard solution.

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of voglibose are not less than 7000 and between 0.8 and 1.2, respectively.

System repeatability: When the test is repeated 6 times with 50 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of voglibose is not more than 3.0 %.

Time span of measurement: About 2.5 times as long as the retention time of voglibose, beginning after the solvent peak

Water Not more than 0.2 % (0.5 g, coulometric titration).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.4 g of Voglibose, dissolve in 80 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination in the same manner and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 26.73 mg $C_{10}H_{21}NO_7$

Containers and Storage ***Containers***—Tight containers.

Voglibose Tables

Voglibose Tablet contains not less than 95.0 % and not more than 105.0 % of the labeled amount of voglibose ($C_{10}H_{21}NO_7$: 267.28).

Method of Preparation Prepare as directed under Tablets, with Voglibose.

Identification Shake vigorously an amount of pulverized Voglibose Tablets, equivalent to 5 mg of voglibose according to the labeled amount, with 40 mL of water, and centrifuge. Transfer the supernatant liquid to a chromatographic column [prepared by pouring 1.0 mL of strongly acidic ion-exchange resin (H type) for column chromatography (100 to 200 μm in particle diameter) into a chromatographic column about 8 mm in internal diameter and about 130 mm in length], and allow to flow at a rate of about 5 mL per minute. Then wash the column with 200 mL of water, and allow to flow with 10 mL of dilute ammonia TS (1 in 4) at a rate of about 5 mL per minute. Filter the eluate two times through a membrane filter with a pore size of not exceeding 0.22 μm. Evaporate the filtrate to dryness at 50 °C under reduced pressure, dissolve the residue with 0.5 mL of a mixture of water and methanol (1 : 1), and use this solution as the test solution. Separately, dissolve 20 mg of Voglibose RS for assay in 2 mL of the mixture of water and methanol (1 : 1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 20 μL each of the test solution and the standard solution on a plate of silicagel for thin-layer chromatography. Develop the plate with a mixture of acetone, ammonia water and water (5 : 3 : 1) to a distance of about 12 cm, air-dry the plate, and allow to stand in iodine vapors: the principal spots from the test solution and the standard solution show a yellow-brown color, and the same R_f value.

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately the mass of not less than 20 Voglibose Tablets, add 80 mL of the mobile phase, and completely disintegrate by shaking. Pipet a volume of this solution, equivalent to about 4 mg of voglibose ($C_{10}H_{21}NO_7$) according to the labeled amount, add the mobile phase to make exactly 100 mL, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size of not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the test solution. Separately, weigh accurately about 20 mg of Voglibose RS (separately determine the water), and dissolve in the mobile phase to make exactly

25 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of voglibose.

$$\text{Amount (mg) of voglibose } (C_{10}H_{21}NO_7) = W_S \times \frac{A_T}{A_S} \times \frac{V}{500}$$

W_S: Amount of voglibose for assay, calculated on the anhydrous basis

Opertating conditions

Apparatus: Use a pumping system for the mobile phase, pumping system for the reaction reagent, sample injection port, column, reaction coil, cooling coil, detector and recording device. Attach a 3-way tube to the outlet for the mobile phase of the column, connect the pumping system for the reaction reagent and the reaction coil, and join outlet of the reaction coil to the spectrofluorometer.

Detector: Spectrofluorometer (excitation wavelength: 350 nm, fluorescence wavelength: 430 nm)

Column: A stainless steel column about 4 mm in internal diameter and about 15 cm in length, packed with aminopropylsilanized silica gel for liquid chromatography (5 μmin particle diameter).

Column temperature: A constant temperature of about 25 °C.

Reaction coil: A polytetrafluoroethylene tube about 0.5 mm in internal diameter and about 20 m in length

Cooling coil: A polytetrafluoroethylene tube about 0.3 mm in internal diameter and about 2 m in length

Mobile phase: To 1.56 g of sodium dihydrogen phosphate hydrate add water to make 500 mL. To this solution add a solution, prepared by dissolving 3.58 g of disodium hydrogen phosphate dodecahydrate in water to make 500 mL, to adjust to pH 6.5. To 300 mL of this solution add 600 mL of acetonitrile.

Reaction reagent: Dissolve 6.25 g of taurine and 2.56 g of sodium periodate in water to make 1000 mL.

Reaction temperature: A constant temperature of about 100 °C.

Cooling temperature: A constant temperature of about 15 °C.

Flow rate of mobile phase : Adjust the flow rate so that the retention time of voglibose is about 20 minutes.

Flow rate of reaction reagent: Same as the flow rate of the mobile phase.

System suitability

System performance: Dissolve 2 mg of Voglibose RS for assay and 0.2 g of lactose hydrate in 5 mL of water, and add the mobile phase to make 50 mL. When the procedure is run with 50 μL of this solution under the above operating conditions, lactose and voglibose are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 50 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak area of voglibose is not more than 2.0 %.

Containers and Storage ***Containers***—Tight containers.

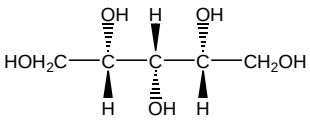
Xenon (^{133}Xe) Injection

Xenon (^{133}Xe) Injection is an aqueous solution for injection containing xenon-133 (^{133}Xe) in the form of solution. Xenon (^{133}Xe) Injection conforms to the requirements of Xenon (^{133}Xe) Injection in the Korean Pharmaceutical Codex.

Description Xenon (^{133}Xe) Injection is a clear, colorless liquid.

Bacterial Endotoxins Less than 175/V EU/mL of Xenon (^{133}Xe) Injection, where V is the maximum recommended dose per mL during the effective time.

Xylitol



$C_5H_{12}O_5$: 152.15

(2*R*,4*S*)-Pentane-1,2,3,4,5-pentol [*87-99-0*]

Xylitol, when dried, contains not less than 98.0 % and not more than 101.0 % of xylitol ($C_5H_{12}O_5$).

Description Xylitol is a white crystalline powder, is odorless and has a sweet taste.
Xylitol is very soluble in water and practically insoluble in ethanol (95).
Xylitol is hygroscopic.

Identification (1) To 1 mL of a solution of Xylitol (1 in 2), add 2 mL of iron (II) sulfate TS and 1 mL of a solution of sodium hydroxide (1 in 5): a blue-green color is observed without turbidity.

(2) Determine the infrared spectra of Xylitol and Xylitol RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavenumbers.

pH Dissolve 5.0 g of Xylitol in 10 mL of freshly boiled and cooled water: the pH of this solution is between 5.0 and 7.0.

Melting Point 93.0 ~ 95.0 °C.

Purity (1) ***Clarity and color of solution***—Dissolve 5 g of Xylitol in 10 mL of water: the solution is clear and colorless.

(2) ***Chloride***—Perform the test with 2.0 g of Xylitol. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.005 %).

(3) ***Sulfate***—Perform the test with 4.0 g of Xylitol. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.006 %).

(4) ***Heavy metals***—Proceed with 4.0 g of Xylitol according to Method 1 and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 5 ppm).

(5) ***Nickel***—Dissolve 0.5 g of Xylitol in 5 mL of water, add 3 drops of dimethylglyoxime TS and 3 drops of ammonia TS and allow to stand for 5 minutes: no red color is produced.

(6) ***Arsenic***—Prepare the test solution with 1.5 g of Xylitol according to Method 1 and perform the test (not more than 1.3 ppm).

(7) ***Sugars***—Dissolve 5.0 g of Xylitol in 15 mL of water, add 4.0 mL of dilute hydrochloric acid and heat on a water-bath for 3 hours under a reflux condenser. After cooling, neutralize with sodium hydroxide TS (indicator: 2 drops of methyl orange TS). Then add water to make 50 mL, transfer 10 mL of this solution to a flask, add 10 mL of water and 40 mL of Fehling's TS, boil gently for 3 minutes and allow to stand to precipitate cuprous oxide. Remove the clear supernatant liquid through a glass filter (G4) and wash the precipitate with warm water until the last washing does not show alkalinity. Filter these washings through the glass filter mentioned above, dissolve the precipitate in the flask in 20 mL of iron (III) sulfate TS, filter the solution through the glass filter mentioned above, wash with water, combine the washings with the filtrate, heat at 80 °C and titrate with 0.1 mol/L potassium permanganate VS: not more than 1.0 mL of 0.1 mol/L potassium permanganate VS is consumed.

(8) ***Related substances (other polyols)***—Weigh accurately 1.0 g of Xylitol, dissolve in water to make 100 mL, and use this solution as the test solution. Separately, weigh accurately 50 mg each of L-Arabinitol RS, Galactitol RS, D-Mannitol RS, and Sorbitol RS, dissolve in water to make 100 mL, and use this solution as the standard solution (1). Separately, weigh accurately 0.5 g of Xylitol RS, dissolve in water to make 25 mL, and use this solution as the standard solution (2). Pipet 1 mL of the standard solution (1) and 5 mL of the standard solution (2), add water to make 10 mL so that each mL contains about 0.05 mg each of L-arabinitol, galactitol, D-mannitol, and sorbitol and about 10 mg of xylitol, and use this solution as the standard solution. Pipet 1.0 mL each of the test solution and standard solution into separate 10 mL round-bottom flasks, add exactly 1.0 mL of the internal standard solution to each flask, and evaporate to dryness with a reflux condenser in a water bath at 60 °C. Add 1 mL of ethanol (99.5), shake carefully, and evaporate to dryness under the same conditions as above. To each residue add exactly 1 mL of pyridine and 1 mL of acetic acid (100), cap each flask, mix for 30 seconds, and allow to stand in a dry oven at 70 °C for 30 minutes. Perform the test with 1 μL each of the test solution and standard solution as directed under Gas Chromatography according to the following conditions: the total amount of related substances is not more than 2.0 %.

$$\text{Amount (\%) of related substances} = 100 \times \frac{C_S}{C_T} \times \frac{Q_T}{Q_S}$$

C_S: Concentration (mg/mL) of each related substance in the standard solution

C_T: Concentration (mg/mL) of Xylitol in the test solution

Q_T: Ratio of the peak area of each derivatized related substance to that of derivatized erythritol obtained from the test solution

Q_S: Ratio of the peak area of each derivatized related substance to that of derivatized erythritol obtained from the standard solution

Internal standard solution—A solution of erythritol (7 in 2000)

Operating conditions

Detector: A hydrogen flame ionization detector

Column: A column about 0.25 mm in internal diameter and about 30 m in length, coated with 14 % cyanopropylphenol-86 % methylpolysiloxane 0.25 μm in thickness.

Column temperature: Maintain at 170 °C for 5 minutes, raise the temperature to 215 °C at the rate of 6 °C per minute, and maintain at 215 °C for 8 minutes. Then raise the temperature to 270 °C at the rate of 10 °C per minute, and maintain at 270 °C for 14 minutes.

Carrier gas: Helium

Flow rate: 1 mL/minute

Injection port temperature: 270 °C

Detector temperature: 280 °C

System suitability

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, the relative retention times of derivatives of erythritol, L-arabinitol, xylitol, D-mannitol, and galactitol with respect to the retention time of derivatized sorbitol are about 0.47, about 0.75, about 0.81, about 0.98 and about 0.99, respectively.

System repeatability: When the test is repeated 5 times with 1 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of xylitol to that of erythritol is not more than 1.5 %.

Loss on Drying Not more than 1.0 % (1 g, in vacu-

um, P_2O_5, 24 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.2 g of Xylitol, previously dried, dissolve in water to make exactly 100 mL. Pipet 10.0 mL of this solution into an iodine flask, add exactly 50 mL of potassium periodate TS and heat on a water-bath for 15 minutes. After cooling, add 2.5 g of potassium iodide, stopper immediately, shake well, allow to stand for 5 minutes in a dark place and titrate with 0.1 mol/L sodium thiosulfate VS (indicaor: 3 mL of starch TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 1.9018 mg of $C_5H_{12}O_5$

Containers and Storage ***Containers***—Tight containers.

Xylitol Injection

Xylitol Injection is an aqueous solution for injection. Xylitol Injection contains less than 95.0 % and not more than 105.0 % of the labeled amount of xylitol ($C_5H_{12}O_5$: 152.15).

Method of Preparation Prepare as directed under Injections, with Xylitol.
No preservative may be added.

Desciption Xylitol Injection is a clear, colorless liquid and has a sweet taste.

Identification Measure a volume of Xylitol Injection, equivalent to 0.1 g of Xylitol according to the labeled amount, add water to make 10 mL and use this solution as the test solution. Separately, dissolve 0.1 g of Xylitol RS in 10 mL of water and use this solution as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin-layer chromatography. Spot 2 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (95), ammonia solution (28) and water (25 : 4 : 3) to a distance of about 10 cm and air dry the plate. Spray evenly silver nitrate-ammonia TS and dry at 105 °C for 15 minutes: the spots from the test solution and the standard solution show a blackish brown color and the same R_f value.

pH 4.5 ~ 7.5.

Sterility Test It meets the requirement.

Bacterial Endotoxins Less than 0.50 EU/mL.

Foreign Insoluble Matter Test It meets the requirement

Insoluble Particulate Matter for Injections Test It meets the requirement.

Determination of Volume of Injection in Containers It meets the requirement.

Assay Measure exactly a volume of Xylitol Injection, equivalent to about 5 g of xylitol ($C_5H_{12}O_5$) according to the labeled amount, add 5.0 mL of the internal standard solution, add water to make exactly 25 mL and use this solution as the test solution. Separately, measure exactly about 0.5g of Xylitol RS after drying in a dessicator (in vacuum, P_2O_5) for 24 hours, add 5.0 mL of the internal standard solution, add water to make exactly 25 mL and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution, as directed under Liquid Chromatography and calculate the ratios, Q_T and Q_S, of the peak area of Xylitol to that of the internal standard for the test solution and the standard solution, respectively.

$$\text{Amount(mg) of xylitol } (C_5H_{12}O_5) = \text{Amount (mg) of Xylitol RS} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of white sugar (5 in 50).

Operating conditions

Detector: A differential refractometer.

Column: A stainless steel column, about 7.8 mm in internal diameter and about 15 to 30 cm in length, packed with strong acidic ion exchange resin (the calcium form of the sulfonated styrene divinylbenzene copolymer, 9 μm in particle diameter).

Column temperature: An ordinary temperature.

Mobile phase: A mixture of acetonitrile and water (75 : 25).

Flow rate: Adjust the flow rate so that the retention time of xylitol is about 5 minutes.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, xylitol and the internal standard are eluted in this order with the resolution between their peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL each of standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of xylitol is not more than 1.0 %.

Containers and Storage ***Containers***—Hermetic containers.

Dried Yeast

Dried Yeast is dried and powdered cells of yeast belonging to *Saccharomyces*.
Dried Yeast contains not less than 0.4 g of protein and not less than 100 μg of thiamine compounds [as thiamine hydrochloride ($C_{12}H_{17}ClN_4OS.HCl$: 337.27)] per 1 g.

Description Dried Yeast is a pale yellow to brown powder and has a characteristic odor and taste.

Identification Dried Yeast, when examined under a microscope, shows isolated cells, spheroidal or oval in shape, and 6 μm to 12 μm in length.

Purity (1) ***Rancidity***—Dried Yeast is free from any unpleasant or rancid odor or taste.

(2) ***Starch***—Add iodine TS to Dried Yeast, and examine microscopically: no or only a few granules are tinted blackish purple.

Loss on Drying Not more than 8.0 % (1 g, 100°C, 8 hours).

Ash Not more than 9.0 % (1 g, proceed as directed in the Ash under the Crude Drugs).

Assay (1) ***Protein*** Weigh accurately about 50 mg of Dried Yeast and perform the test as directed under the Nitrogen Determination.

$$\text{Amount(mg) of protein in 1 g of Dried Yeast} = \text{Amount (mg) of nitrogen (N)} \times 6.25 \times \frac{1}{\text{amount (g) of sample}}$$

(2) ***Thiamine*** Weigh accurately about 1 g of Dried Yeast, add 1 mL of dilute hydrochloric acid and 80 mL of water, and heat in a water-bath at 80 ℃ to 85 ℃ for 30 minutes with occasional shaking. After cooling, add water to make exactly 100 mL, and centrifuge for 10 minutes. Pipet 4.0 mL of the clear supernatant liquid, add exactly 5 mL of acetic acid-sodium acetate TS and exactly 1 mL of enzyme TS, and allow to stand at 45 ~ 50 ℃ for 3 hours. Place exactly 2 mL of this solution onto a chromatographic column prepared by pouring 2.5 mL of a weakly acidic CM-bridged cellulose cation exchanger (H type) (40 μm to 110 μm in particle diameter) into a chromatographic tube about 1 cm in internal diameter and about 17 cm in length, and elute at the flow of about 0.5 mL per minute. Wash the upper part of the column with a small amount of water, and wash the column with two 10 mL portions of water at the flow rate of about 1 mL per minute. Elute the column with two 2.5 mL portions of diluted phosphoric acid (1 in 50) at the flow rate of about 0.5 mL of per minute, and combine the eluate. To a eluate add 1.0 mL of the internal standard solution and 10 mg of sodium 1-octanesulfonate, and after dissolving, use this solution as the test solution. Separately, weigh accurately about 15 mg of the Thiamine Hydrochloride RS (determine the water content in the same manner as for Thiamine Hydrochloride), dissolve in 0.001 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 1.0 mL of this solution, and add the mobile phase to make exactly 100 mL. Pipet 1.0 mL of this solution, and add 1.0 mL of the internal standard solution and 3.0 mL of the mobile phase, and use this solution as the standard solution. Perform the test with 200 μL each of the test solution and the standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of thiamine to that of the internal standard.

$$\text{Amount (μg) of thiamine in 1 g of Dried Yeast} = \text{Amount (mg) of Thiamine Hydrochloride RS, calculated on the anhydrous basis} \times \frac{Q_T}{Q_s} \times \frac{1}{\text{amount (g) of the sample}} \times 12.5$$

Internal standard solution Dissolve 0.01 g of phenacetin in acetonitrile to make 100 mL, and to 1 mL of this solution add diluted acetonitrile (1 in 5) to make 100 mL.

Operating conditions
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in internal diameter and about 15 cm to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm to 10 μm in particle diameter).

Column temperature: A constant temperature at about 40 °C.

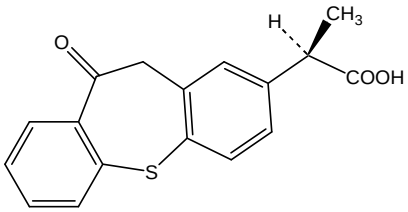
Mobile phase: Dissolve 2.7 g of potassium dihydrogenphosphate in 1000 mL of water, and adjust the pH to 3.5 with diluted phosphoric acid (1 in 10). Dissolve 1.6 g of sodium 1-octanesulfonate in 800 mL of this solution, and add 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of thiamine is about 8 minutes.

Selection of column: Proceed with 200 μL of the standard solution under the above operating conditions. Use a column giving elution of thiamine and the internal standard in this order with the resolution between these peaks being not less than 8.0.

Containers and Storage ***Containers***—Tight containers.

Zaltoprofen



and enantiomer

$C_{17}H_{14}O_3S$: 298.36

2-(10-Oxo-10,11-dihydrodibenzo[b,f]thiepin-2-yl)propanoic acid [*74711-43-6*]

Zaltoprofen, when dried, contains not less than 99.0 % and not more than 101.0 % of zaltoprofen ($C_{17}H_{14}O_3S$).

Description Zaltoprofen appears as white to light yellow crystals or crystalline powder.
Zaltoprofen is freely soluble in acetone, soluble in methanol or in ethanol (99.5), and practically insoluble in water.
Zaltoprofen is gradually decomposed by light.
A solution of Zaltoprofen in acetone (1 in 10) shows no optical rotation.

Identification (1) To 0.2 g of Zaltoprofen add 0.5 g of sodium hydroxide, heat gradually, and carbonize. After cooling, add 5 mL of diluted hydrochloric acid (1 in 2): the gas evolved darkens moist lead acetate paper.

(2) Determine the absorption spectra of solutions of Zaltoprofen and Zaltoprofen RS in ethanol (99.5) (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared spectra of Zaltoprofen and Zaltoprofen RS as directed in the potassium bromide disk method under Infrared Spectrophotometry: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting Point 135 ~ 139 °C.

Purity (1) ***Heavy metals***—Proceed with 2.0 g of Zaltoprofen according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(2) ***Arsenic***—Prepare the control solution with 1.0 g of Zaltoprofen according to Method 3, and perform the test. Use a solution of magnesium nitrate hexahyrate in ethanol (95) (2 in 25) (not more than 2 ppm).

(3) ***Related substances***—Dissolve 50 mg of Zaltoprofen in 50 mL of the mobile phase, and use this solution as the test solution. Pipet 1 mL of the test solution, and add the mobile phase to make exactly 50 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine the peak areas of each solution by the automatic integration method: the area of the peak other than zaltoprofen and the peak having the relative retention time of about 0.7 with respect to zaltoprofen from the test solution is not larger than the peak area of zaltoprofen from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 240 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilaznied silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile p hase: A mixture of acetonitrile, water, and acetic acid (100) (300 : 200 : 1)

Flow rate: Adjust the flow rate so that the retention time of zaltoprofen is about 4 minutes.

System suitability

Test for required detectability: Pipet 2 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area obtained from 20 μL of this solution is equivalent to 8 to 12 % of that of zaltoprofen from the standard solution.

System performance: Dissolve 25 mg of zaltoprofen and 50 mg of isopropyl benzoate in 100 mL of ethanol (99.5). To 1 mL of this solution add the mobile phase to make exactly 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, zaltoprofen and isopropyl benzoate are eluted in this order with the resolution between these peaks being not less than 6.0.

System repeatability: When the test is repeated 6 times with 20 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of zaltoprofen is not more than 2.0 %.

Time span of measurement: About 15 times as long as the retention time of zaltoprofen beginning after the solvent peak.

Loss on Drying Not more than 0.5 % (1 g, 105 °C, 4 hours).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.5 g of Zaltoprofen, dissolve in 50 mL of methanol, and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 29.84 mg of $C_{17}H_{14}O_3S$

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Zaltoprofen Tablets

Zaltoprofen Tablets contain not less than 95.0 % and not more than 105.0 % of the labeled amount of zaltoprofen ($C_{17}H_{14}O_3S$: 298.36).

Method of Preparation Prepare as directed under Tablets, with Zaltoprofen.

Identification To an amount of powdered Zaltoprofen Tablets, equivalent to 80 mg of zaltoprofen according to the labeled amount, add 30 mL of ethanol (99.5), shake well, and centrifuge. To 1 mL of the clear supernatant liquid add ethanol (99.5) to make 20 mL. To 2 mL of this solution add ethanol (99.5) to make 25 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 227 and 231 nm and between 329 and 333 nm, and a shoulder between 241 and 245 nm.

Dissolution Test Perform the test with 1 tablet of Zaltoprofen Tablets at 50 revolutions per minute according to Method 2, using 900 mL of the 2nd fluid for dissolution test as the dissolution solution. Take not less than 20 mL of the dissolved solution 30 minutes after the start of the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the 2nd fluid for dissolution test to make V' mL so that each mL contains about 44 μg of zaltoprofen ($C_{17}H_{14}O_3S$), and use this solution as the test solution. Separately, weigh accurately about 22 mg of Zaltoprofen RS, previously dried at 105 °C for 4 hours, dissolve in 20 mL of ethanol (99.5), and add the 2nd fluid for dissolution test to make exactly 100 mL. Pipet 4 mL of this solution, add the 2nd fluid for dissolution test to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 340 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry, using the dissolution solution as the blank. The dissolution rate of Zaltoprofen Tablets in 30 minutes is not less than 75 %.

$$\text{Dissolution rate (\%) with respect to the labeled amount of zaltoprofen } (C_{17}H_{14}O_3S) = \text{Amount (mg) of Zaltoprofen RS} \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 180$$

C: Labeled amount (mg) of zaltoprofen ($C_{17}H_{14}O_3S$) in 1 tablet

Uniformity of Dosage Units It meets the requirement.

Assay Weigh accurately the mass of not less than 10 Zaltoprofen Tablets, add 40 mL of water, and shake well to disintegrate. Add ethanol (95), shake well, and add ethanol (95) to make exactly 200 mL, and centrifuge. Pipet a volume of the clear supernatant liquid, equivalent to about 8 mg of zaltoprofen ($C_{17}H_{14}O_3S$), add exactly 10 mL of the internal standard solution and ethanol (95) to make 50 mL, and use this solution as the test solution. Separately, weigh accurately about 80 mg of Zaltoprofen RS, previously dried at 105 °C for 4 hours, add 4 mL of water, and add ethanol (95) to make exactly 20 mL. To 2 mL of this solution add exactly 10 mL of the internal standard solution and ethanol (95) to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of zaltoprofen to that of the internal standard.

$$\text{Amount (mg) of zaltoprofen } (C_{17}H_{14}O_3S) = \text{Amount (mg) of Zaltoprofen RS} \times \frac{Q_T}{Q_S} \times \frac{1}{10}$$

Internal standard solution—A solution of benzyl benzoate in acetonitrile (1 in 1000)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 240 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: A mixture of acetonitrile, water, and acetic acid (100) (300 : 200 : 1)

Flow rate: Adjust the flow rate so that the retention time of zaltoprofen is about 4 minutes.

System suitability

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, zaltoprofen and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of ratios of the peak area of zaltoprofen to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Tight containers.

Zinc Chloride

$ZnCl_2$: 136.32

Dichlorozinc [*7646-85-7*]

Zinc Chloride contains not less than 97.0 % and not more than 101.0 % of zinc chloride ($ZnCl_2$).

Description Zinc Chloride is a white, crystalline powder, rods or masses and is odorless.
Zinc Chloride is very soluble in water and freely soluble in ethanol (95). Its solution may sometimes be slightly turbid and the solution becomes clear on addition of a small amount of hydrochloric acid.

pH—The pH of an aqueous solution of Zinc Chloride (1 in 2) is between 3.3 and 5.3.
Zinc Chloride is deliquescent.

Identification A solution of Zinc Chloride (1 in 30) responds to the Qualitative Tests for zinc salt and for chloride.

Purity (1) ***Clarity and color of solution***—Dissolve about 1.0 g of Zinc Chloride in 10 mL of water and 2 drops of hydrochloric acid: the solution is clear and colorless.

(2) ***Oxychloride***—Add 0.25 g of Zinc Chloride to 5 mL of water and 5 mL of ethanol (95), shake gently, and add 0.30 mL of 1 mol/L hydrochloric acid: the solution is clear.

(3) ***Sulfate***—Perform the test with 2.0 g of Zinc Chloride. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010 %).

(4) ***Ammonium***—Dissolve 0.5 g of Zinc Chloride in 5 mL of water and warm with 10 mL of a solution of sodium hydroxide (1 in 6): the evolving gas does not change moistened red litmus paper to blue.

(5) ***Heavy metals***—Dissolve 0.5 g of Zinc Chloride in 5 mL of water in a Nessler tube, shake thoroughly with 15 mL of potassium cyanide TS, add 1 drop of sodium sulfide TS, allow to stand for 5 minutes and immediately observe from the top downward against a white background: the solution has no more color than the following control solution (not more than 50 ppm).

Control solution—To 2.5 mL of standard lead solution, add 3 mL of water and 15 mL of potassium cyanide TS, shake thoroughly and add 1 drop of sodium sulfide TS.

(6) ***Alkali earth metals and alkali metal***—Dissolve 2.0 g of Zinc Chloride in 120 mL of water, add ammonium sulfide TS to complete precipitation and add water to make 200 mL. shake thoroughly and filter through dry filter paper. Discard the first 20 mL of the filtrate, take the following 100 mL of the filtrate, evaporate with 3 drops of sulfuric acid to dryness and heat the residue strongly at 600 °C to constant mass: the weight is not more than 10.0 mg.

(7) ***Arsenic***—Prepare the test solution with 0.40 g of Zinc Chloride according to Method 1 and perform the test (not more than 5 ppm).

Assay Weigh accurately about 0.3 g of Zinc Chloride and add 0.4 mL of dilute hydrochloric acid and water to make exactly 200 mL. Measure exactly 20 mL of the solution, add 80 mL of water, 2 mL of ammonia-ammonium chloride buffer solution, pH 10.7 and titrate with 0.01 mol/L disodium ethylenediaminetetraacetate VS (indicator: 40 mg of eriochrome black T-sodium chloride indicator).

Each mL of 0.01 mol/L
disodium ethylenediaminetetraacetate VS
= 1.3630 mg of $ZnCl_2$

Containers and Storage ***Containers***—Tight containers.

Zinc Oxide

ZnO: 81.41

[*1314-13-2*]

Zinc Oxide, when ignited, contains not less than 99.0 % and not more than 101.0 % of zinc oxide (ZnO).

Description Zinc Oxide is a white, amorphous powder, is odorless and tasteless.
Zinc Oxide is practically insoluble in water, in acetic acid (100), in ethanol (95) or in ether.
Zinc Oxide dissolves in dilute hydrochloric acid or in sodium hydroxide TS.
Zinc Oxide gradually absorbs carbon dioxide from air.

Identification (1) Ignite Zinc Oxide: a yellow color is observed and disappears on cooling.

(2) A solution of Zinc Oxide in dilute hydrochloric acid (1 in 10) responds to the Qualitative Tests for zinc salt.

Purity (1) ***Carbonate and clarity and color of solution***—To 2.0 g of Zinc Oxide, add 10 mL of water, mix with shaking, add 30 mL of dilute sulfuric acid and heat in a water-bath with stirring: no effervescence occurs and the solution obtained is clear and colorless.

(2) ***Alkali***—To 1.0 g of Zinc Oxide, add 10 mL of water and boil for 2 minutes. After cooling, filter through a glass filter (G3) and to the filtrate, add 2 drops of phenolphthalein TS and 0.20 mL of 0.1 mol/L hydrochloric acid VS: the solution is colorless.

(3) ***Sulfate***—To 0.5 g of Zinc Oxide, add 40 mL of water, mix with shaking and filter. To 20 mL of the filtrate, add 1 mL of dilute hydrochloric acid and water to make 50 mL and perform the test using this solution as the test solution. Prepare the control solution with

0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.096 %).

(4) ***Iron***—Dissolve 1.0 g of Zinc Oxide in 50 mL of dilute hydrochloric acid (1 in 2), dissolve 0.1 g of ammonium peroxydisulfate in this solution and extract with 20 mL of 4-methyl-2-pentanone. Add 30 mL of acetic acid-sodium acetate buffer solution for Iron Limit Test, pH 4.5, to the 4-methyl-2-pentanone layer, extract again and use the layer of the buffer solution as the test solution. Separately, perform the test in the same manner with 1.0 mL of standard iron solution and use the layer so obtained as the control solution. Add 2 mL each of ascorbic acid solution for Iron Limit Test (1 in 100) to the test solution and the control solution, respectively, mix, allow to stand for 30 minutes, add 5 mL of an ethanol solution of α,α'-dipyridyl (1 in 200) and water to make 50 mL. After allowing to stand for 30 minutes, compare the color of the both liquids against a white backgound: the color of the liquid from the test solution is not stronger than that from the control solution (not more than 10 ppm).

(5) ***Cadmium***—Dissolve 2.0 g of Zinc Oxide in 14 mL of diluted dilute nitric acid (1 in 2), boil for 1 minute, cool, add water to make exactly 100 mL, and use this solution as the test solution. Separately, mix the standard cadmium solution and 3.5 % nitric acid, and use this solution as the standard solution. Determine the absorbances of the test solution and standard solution as directed under Atomic Absorption Spectrophotometry according to the following conditions: the absorbance of the test solution is not more than that of the standard solution (not more than 10 ppm).

Gas: Dissolved acetylene or propane – Air
Lamp: Cadmium hollow cathode lamp
Wavelength: 228.8 nm

(6) ***Lead***—To 2.0 g of Zinc Oxide, add 20 mL of water, then add 5 mL of acetic acid (100) with stirring and heat in a water-bath until dissolved completely. Cool and add 5 drops of potassium chromate TS: no turbidity is produced.

(7) ***Arsenic***—Dissolve 0.5 g of Zinc Oxide in 5 mL of dilute hydrochloric acid, use this solution as the test solution and perform the test (not more than 4 ppm).

Loss on Ignition Not more than 1.0 % (1 g, 850 °C, 1 hour).

Assay Weigh accurately about 0.8 g of Zinc Oxide, previously ignited at 850 °C for 1 hour, dissolve in 2 mL of water and 3 mL of hydrochloric acid and add water to make exactly 100 mL. Pipet 10.0 mL of this solution, add 80 mL of water, then add a solution of sodium hydroxide (1 in 50) until a slight precipitate is produced. Add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7 and titrate with 0.05 mol/L disodium ethylenediaminetetraacetate VS (indicator: 40 mg of eriochrome black T-sodium chloride indicator).

Each mL of 0.05 mol/L
disodium ethylenediaminetetraacetate VS
= 4.069 mg of ZnO

Containers and Storage ***Containers***—Tight containers.

Zinc Sulfate Hydrate

$ZnSO_4 \cdot 7H_2O$: 287.58

[*7446-20-0*]

Zinc Sulfate Hydrate contains not less than 99.0 % and not more than 102.0 % of zinc sulfate hydrate ($ZnSO_4 \cdot 7H_2O$).

Description Zinc Sulfate Hydrate appears as colorless crystals or white crystalline powder.
Zinc Sulfate Hydrate is very soluble in water and practically insoluble in ethanol (99.5) or ether.
Zinc Sulfate hydrate effloresces in dry air.

Identification (1) A solution of Zinc Sulfate Hydrate (1 in 20) responds to the Qualitative Tests for zinc salt.

(2) A solution of Zinc Sulfate (1 in 20) responds to the Qualitative Tests for sulfate.

pH Dissolve 1.0 g of Zinc Sulfate in 20 mL of water: the pH of this solution is between 4.4 and 6.0.

Purity (1) ***Clarity and color of solution***—Dissolve 0.25 g of Zinc Sulfate in 5 mL of water: the solution is clear and colorless.

(2) ***Heavy metals***—Dissolve 1.0 g of Zinc Sulfate Hydrate in 10 mL of water contained in a Nessler tube. Add 20 mL of potassium cyanide TS and mix well. Add 2 drops of sodium sulfide TS and allow the mixture to stand for 5 minutes. Observe vertically against a white background, the color of the solution is not more intense than the following control solution (not more than 10 ppm).

Control solution—To 1.0 mL of standard lead solution, add 10 mL of water and 20 mL of potassium cyanide TS and mix well. Add 2 drops of sodium sulfide TS.

(3) ***Alkalies and alkaline earths***—Dissolve 2.0 g of Zinc Sulfate Hydrate in 150 mL of water, add a suitable amount of ammonium sulfide TS to complete the precipitation and add water to make exactly 200 mL. Shake well and filter through a dry filter paper. Discard the first 20 mL of the filtrate, take exactly 100 mL of the subsequent filtrate, evaporate to dryness and ignite as directed under the Residue on ignition: the mass of the residue is not more than 5.0 mg.

(4) ***Arsenic***—Prepare the test solution with 0.10 g

of Zinc Sulfate Hydrate according to Method 1 and perform the test (not more than 2 ppm).

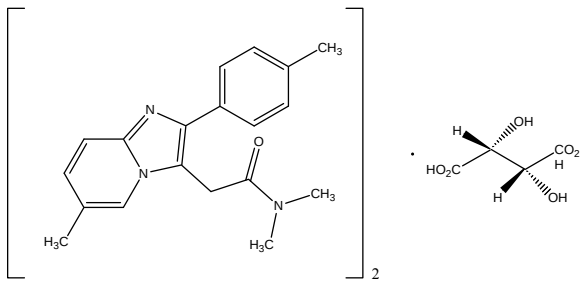
Loss on Drying 35.5 ~ 38.5 % (1.0 g, 105 °C, 3 hours).

Assay Weigh accurately about 0.3 g of Zinc Sulfate Hydrate and dissolve in water to make exactly 100 mL. Measure exactly 25 mL of this solution, add 100 mL of water and 2 mL of ammonia-ammonium chloride buffer solution, pH 10.7 and titrate with 0.01 mol/L disodium ethylenediaminetetraacetate VS (indicator: 40 mg of eriochrome black T-sodium chloride indicator).

Each mL of 0.01 mol/L
disodium ethylenediaminetetraacetate VS
= 2.8756 mg of $ZnSO_4 \cdot 7H_2O$

Containers and Storage ***Containers***—Tight containers.

Zolpidem Tartrate



$C_{42}H_{48}N_6O_8$: 764.87

2,3-Dihydroxybutanedioic acid; *N,N*-dimethyl-2-[6-methyl-2-(4-methylphenyl)imidazo[1,2-a]pyridin-3-yl]acetamide [*99294-93-6*]

Zolpidem Tartrate contains not less than 98.5 % and not more than 101.0 % of zolpidem tartrate ($C_{42}H_{48}N_6O_8$), calculated on the anhydrous basis.

Description Zolpidem Tartrate is a white crystalline powder.
Zolpidem Tartrate is sparingly soluble in methanol, slightly soluble in water, and practically insoluble in dichloromethane.
Zolpidem Tartrate is hygroscopic.

Identification (1) Dissolve 0.10 g each of Zolpidem Tartrate and Zolpidem Tartrate RS in 10 mL of 0.1 mol/L hydrochloric acid TS and add 10 mL of water. Add dropwise 1 mL of 3.4 w/v % ammonia water with stirring. Filter and collect the resulting precipitate. Wash each precipitate with water and then dry at 100 to 105 °C for 2 hour to obtain test precipitate and reference precipitate, respectively. Determine the infrared spectra of the precipitates as directed in the potassium bromide disk method under Infrared Spectrophotometry, respectively: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Dissolve 50 mg of Zolpidem Tartrate in 5 mL of methanol, add 0.1 mL of diethylamine and methanol to make 10 mL, and use this solution as the test solution. Separately, dissolve 50 mg of Zolpidem Tartrate RS in 5 mL of methanol, add 0.1 mL of diethylamine and methanol to make 10 mL, and use this solution as the standard solution (1). Also dissolve 50 mg of Flunitrazepam RS in dichloromethane to make 10 mL. Mix 1 mL of the solution with 1 ml of the standard solution (1), and use this solution as the standard solution (2). Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the test solution and the standard solutions (1) and (2) on a plate of silica gel with a fluorescent indicator for thin-layer chromatography. Develop the plate with the mixture of ethyl acetate, cyclohexane and diethylamine (45 : 45 : 10) to a distance of about 12 cm and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm). The R_f value of principal spot in the chromatogram obtained with the test solution is the same as that of the principal spot in the chromatogram from the standard solution (1). The test is not valid unless the chromatogram from the standard solution (2) shows two clearly separated spots.

(3) Dissolve about 0.1 g of Zolpidem Tartrate in 1 mL of warm methanol. 0.1 mL of the solution responds to the Qualitative Test for 3) of tartrates.

Purity (1) ***Clarity and color of solution***—Mix 0.25 g of Zolpidem Tartrate with 0.125 g of L-tartaric acid, dissolve the mixture in 20 mL of water and add to make 25 mL. The solution is clear

(2) ***Heavy metals***—Proceed with 2.0 g of Zolpidem Tartrate according to Method 4 under Heavy Metals Limit Test, and perform the test. Prepare the control solution with 2.0 mL of standard lead solution (not more than 10 ppm).

(3) ***Related substances***—Dissolve 10 mg of Zolpidem Tartrate in 20 mL of methanol, and use this solution as the test solution. Pipet 1 mL of this solution, and add methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area of each solution by the automatic integration method: the total area of the peaks other than zolpidem from the test solution is not larger than the peak area of zolpidem from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4.6 mm in internal diameter and about 7.5 cm in length, packed

with octadecylsilanized silica gel for Liquid Chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: A mixture of a solution prepared by adding 1000 mL of water to 4.9 g of phosphoric acid and adjusting the pH to 5.5 with triethylamine, methanol, and acetonitrile (11 : 5 : 4)

Flow rate: Adjust the flow rate so that the retention time of zolpidem is about 5 minutes.

System suitability

System performance: Dissolve 10 mg each of Zolpidem Tartrate and benzyl paraoxybenzoate in 100 mL of methanol. When the procedure is run with 5 μL of this solution under the above operating conditions, zolpidem and benzyl paraoxybenzoate are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of zolpidem is not more than 5.0 %.

Time span of measurement: About 5 times as long as the retention time of zolpidem

Water not more than 3.0 % (0.5 g, volumetric titration, direct titration).

Residue on Ignition Not more than 0.1 % (1 g).

Assay Weigh accurately about 0.30 g of Zolpidem Tartrate and dissolve in a mixture of 20 mL of acetic acid (100) and 20 mL of acetic anhydride. Titrate with 0.1 mol/L perchloric acid VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Carry out a blank titration.

Each ml of 0.1 mol/L perchloric acid VS
= 38.24 mg of $C_{42}H_{48}N_6O_8$.

Containers and Storage ***Containers***—Tight containers.

Storage—Light-resistant.

Zolpidem Tartrate Tablets

Zolpidem Tartrate Tablets contain not less than 95.0 % and not more than 105.0 % of the labeled amount of zolpidem tartrate ($C_{42}H_{48}N_6O_8$: 764.87).

Method of Preparation Prepare as directed under Tablets, with Zolpidem Tartrate.

Identification To 1 tablet of Zolpidem Tartrate tablets add 100 mL of 0.1 mol/L hydrochloric acid TS, mix well for 30 minutes, and filter. Discard the first 20 mL of the filtrate, and to a volume of the subsequent filtrate, equivalent to 1 mg of zolpidem tartrate according to the labeled amount, add 0.1 mol/L hydrochloric acid to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry: it exhibits maxima between 235 and 239 nm and between 292 and 296 nm.

Dissolution Test Perform the test with 1 tablet of Zolpidem Tartrate Tablets at 50 revolutions per minute according to Method 2 under Dissolution Test, using 900 mL of water as the dissolution solution. Take not less than 20 mL of the dissolved solution 15 minutes after the start of the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the 2nd fluid for dissolution test to make exactly V' mL so that each mL contains about 2.8 μg of zolpidem tartrate according to the labeled amount, and use this solution as the test solution. Separately, weigh accurately about 22 mg of Zolpidem Tartrate RS (separately determine the water in the same manner as Zolpidem Tartrate Tablets), and dissolve in water to make exactly 100 mL. To 5 mL of this solution add water to make exactly 200 mL. To 25 mL of this solution add the 2nd solution or dissolution test to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 242 nm of the test solution and standard solution as directed under Ultraviolet-visible Spectrophotometry, using the diluted 2nd solution for dissolution test (1 in 2) as the blank. The dissolution rate of Zolpidem Tartrate Tablets in 15 minutes is not less than 80 %.

Dissolution rate (%) with respect to the labeled amount of zolpidem tartrate ($C_{42}H_{48}N_6O_8$)
= Amount (mg) of Zolpidem Tartrate RS,
calculated on the anhydrous basis $\times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times \frac{45}{4}$

C: Labeled amount (mg) of zolpidem tartrate ($C_{42}H_{48}N_6O_8$) in 1 tablet

Uniformity of Dosage Units Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Zolpidem Tartrate Tablets add V/10 mL of 0.1 mol/L hydrochloric acid TS, and shake well for 15 minutes to disintegrate. To this solution add 2V/5 mL of methanol and exactly V/10 mL of the internal standard solution, shake well for 15 minutes, and add methanol to make V mL so that each mL contains about 0.1 mg of zolpidem tartrate ($C_{42}H_{48}N_6O_8$). Centrifuge, and use the clear supernatant liquid as the test solution. Separately, weigh accurately about 25 mg of Zolpidem Tartrate RS (separately determine the water in the same manner as Zolpidem Tartrate Tablets), dissolve in 25 mL of 0.1 mol/L hydrochloric acid TS, add exactly 25 mL of internal standard solution and methanol to make 250 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and standard solution as directed under Liquid

Chromatography according to the same conditions as the Assay.

$$\text{Amount (mg) of zolpidem tartrate } (C_{42}H_{48}N_6O_8) = \text{Amount (mg) of Zolpidem Tartrate RS, calculated on the anhydrous basis} \times \frac{Q_T}{Q_S} \times \frac{V}{500}$$

Internal standard solution—A solution of benzyl parahydroxybenzoate in methanol (1 in 1000)

Assay To not less than 20 Zolpidem Tartrate Tablets add *V*/10 mL of 0.1 mol/L hydrochloric acid TS, and shake well for 15 minutes to disintegrate. Add exactly 2*V*/5 mL of methanol and *V*/10 mL of the internal standard solution, mix well for 15 minutes, and add methanol to make *V* mL so that each mL contains about 1 mg of zolpidem tartrate. Centrifuge, and to 1 mL of the clear supernatant liquid add a mixture of methanol and 0.1 mol/L hydrochloric acid TS (9 : 1) to make 10 mL, and use this solution as the test solution. Separately, weigh accurately about 25 mg of Zolpidem Tartrate RS (separately determine the water in the same manner as Zolpidem Tartrate Tablets), dissolve in 0.1 mol/L hydrochloric acid TS, add exactly 2.5 mL of the internal standard solution and methanol to make 250 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the test solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of zolpidem to that of the internal standard.

$$\text{Amount (mg) of zolpidem tartrate } (C_{42}H_{48}N_6O_8) \text{ in 1 tablet} = \text{Amount (mg) of Zolpidem Tartrate RS, calculated on the anhydrous basis} \times \frac{Q_T}{Q_S} \times \frac{V}{500}$$

Internal standard solution—A solution of benzyl parahydroxybenzoate in methanol (1 in 100)

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 254 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 7.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25 °C

Mobile phase: A mixture of a solution prepared by adding 1000 mL of water to 4.9 g of phosphoric acid and adjusting the pH to 5.5 with triethylamine, methanol, and acetonitrile (55 : 25 : 20)

Flow rate: Adjust the flow rate so that the retention time of zolpidem is about 5 minutes.

System suitability

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, zolpidem and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 5 μL each of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of zolpidem to that of the internal standard is not more than 1.0 %.

Containers and Storage ***Containers***—Well-closed containers.