fire, and preferably in a cold place.

Pyrrolnitrin

ピロールニトリン



C₁₀H₆Cl₂N₂O₂: 257.07 3-Chloro-4-(3-chloro-2-nitrophenyl)pyrrole [*1018-71-9*]

Pyrrolnitrin contains not less than 970 μ g (potency) and not more than 1020 μ g (potency) per mg, calculated on the dried basis. The potency of Pyrrolnitrin is expressed as mass (potency) of pyrrolnitrin (C₁₀H₆Cl₂N₂O₂).

Description Pyrrolnitrin occurs as yellow to yellow-brown, crystals or crystalline powder.

It is freely soluble in methanol and in ethanol (95), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Pyrrolnitrin in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pyrrolnitrin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pyrrolnitrin as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Pyrrolnitrin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 124 – 128°C

Purity Related substances—Dissolve 0.10 g of Pyrrolnitrin in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample 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 these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of xylene, ethyl acetate and formic acid (18:2:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly diluted sulfuric acid (1 in 3) on the plate, and heat at 100°C for 30 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Conduct this procedure using light-resistant vessels. Weigh accurately an amount of Pyrrolnitrin and Pyrrolnitrin RS, equivalent to about 50 mg (potency) each, and dissolve separetely in diluted acetonitrile (3 in 5) to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 10 mL of the internal standard solution, add diluted acetonitrile (3 in 5) to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with $5 \,\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of pyrrolnitrin to that of the internal standard.

Amount [μ g (potency)] of C₁₀H₆Cl₂N₂O₂ = $M_{\rm S} \times Q_{\rm T}/Q_{\rm S} \times 1000$

M_S: Amount [mg (potency)] of Pyrrolnitrin RS

Internal standard solution—A solution of benzyl benzoate in diluted acetonitrile (3 in 5) (3 in 500).

Operating conditions-

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

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

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

Mobile phase: A mixture of water and acetonitrile (11:9). Flow rate: Adjust the flow rate so that the retention time of pyrrolnitrin is about 9 minutes.

System suitability-

System performance: When the procedure is run with $5 \mu L$ of the standard solution under the above operating conditions, pyrrolnitrin 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 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pyrrolnitrin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Quinapril Hydrochloride

キナプリル塩酸塩



C₂₅H₃₀N₂O₅.HCl: 474.98 (3*S*)-2-((2*S*)-2-{[(1*S*)-1-Ethoxycarbonyl-

3-phenylpropyl]amino}propanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid monohydrochloride [82586-55-8]

Quinapril Hydrochloride contains not less than 99.0% and not more than 101.0% of $C_{25}H_{30}N_2O_5$.

HCl, calculated on the anhydrous basis.

Description Quinapril Hydrochloride occurs as a white powder.

It is very soluble in methanol, freely soluble in water and in ethanol (99.5), and soluble in acetic acid (100).

It is deliquescent.

Identification (1) Determine the absorption spectrum of a solution of Quinapril Hydrochloride in methanol (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Quinapril Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Quinapril Hydrochloride (1 in 20) responds to the Qualitative Tests $\langle 1.09 \rangle$ for chloride.

Optical rotation $\langle 2.49 \rangle$ [α]²⁰_D: +14.4 - +16.0° (0.5 g, calculated on the anhydrous basis, methanol, 25 mL, 100 mm).

Purity (1) Heavy metals $\langle 1.07 \rangle$ —Proceed with 1.0 g of Quinapril 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 50 mg of Quinapril Hydrochloride in 50 mL of a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly $10 \,\mu L$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peaks, having the relative retention time of about 0.5 and about 2.0 to quinapril, obtained from the sample solution are not larger than the peak area of quinapril from the standard solution, respectively, the area of peak other than quinapril and above mentioned peak from the sample solution are not larger than 2/5 times the peak area of quinapril from the standard solution, and the total area of the peaks other than quinapril from the sample solution is not larger than 3 times the peak area of quinapril from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about $25^{\circ}C$.

Mobile phase: While keeping the temperature not below 25° C, adjust to pH 2.0 of 0.2 mol/L potassium dihydrogen phosphate TS with perchloric acid. To 1000 mL of this solution add 1000 mL of acetonitrile for liquid chromatography.

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

Time span of measurement: About 4 times as long as the retention time of quinapril, beginning after the solvent peak. *System suitability*—

Test for required detectability: Pipet 10 mL of the standard solution, and add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly 100 mL. Confirm that the peak area of quinapril obtained with 10 μ L of this solution is equivalent to 7 to 13% of that with 10 μ L of the standard solution.

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of quinapril are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of quinapril is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Water $\langle 2.48 \rangle$ Not more than 1.0% (0.2 g, coulometric titration).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Start to titrate within 3 minutes after dissolving Quinapril Hydrochloride. Weigh accurately about 0.5 g of Quinapril Hydrochloride, dissolve in 70 mL of acetic acid (100), add 4 mL of bismuth nitrate TS, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = $47.50 \text{ mg of } C_{25}H_{30}N_2O_5.HCl$

Containers and storage Containers—Tight containers. Storage—In a cold place.

Quinapril Hydrochloride Tablets

キナプリル塩酸塩錠

Quinapril Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of quinapril hydrochloride ($C_{25}H_{30}N_2O_5$.HCl: 474.98).

Method of preparation Prepare as directed under Tablets, with Quinapril Hydrochloride.

Identification To a quantity of powdered Quinapril Hydrochloride Tablets, equivalent to 20 mg of Quinapril Hydrochloride according to the labeled amount, add 10 mL of methanol, shake for 5 minutes, and centrifuge. To 5 mL of the supernatant liquid add 0.5 mL of dilute hydrochloric acid, and add methanol to make 20 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: it exhibits maxima between 256 nm and 260 nm, between 262 nm and 266 nm, and between 269 nm and 273 nm.

Purity To an amount of the supernatant liquid obtained in the Assay add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) so that

each mL contains 0.2 mg of Quinapril Hydrochloride according to the labeled amount, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly $10 \,\mu L$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak having the relative retention time of about 0.5 to quinapril obtained from the sample solution is not larger than 2 times the peak area of quinapril from the standard solution, and the area of the peak, having the relative retention time of about 2.0 to quinapril obtained from the sample solution is not larger than the peak area of quinapril from the standard solution.

Operating conditions—

Proceed as directed in the operating conditions in the Purity (2) under Quinapril Hydrochloride.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of quinapril are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of quinapril is not more than 2.0%.

Uniformity of dosage units $\langle 6.02 \rangle$ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Quinapril Hydrochloride Tablets add 3V/5 mL of a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1), shake vigorously to disintegrate the tablet, shake again for 10 minutes, add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly V mL so that each mL contains about 0.22 mg of quinapril hydrochloride (C₂₅H₃₀N₂O₅.HCl), and centrifuge. Pipet 15 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make solution. Then, proceed as directed in the Assay.

- Amount (mg) of quinapril hydrochloride ($C_{25}H_{30}N_2O_5$.HCl) = $M_S \times Q_T/Q_S \times V/120$
 - $M_{\rm S}$: Amount (mg) of quinapril hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) (1 in 800).

Dissolution $\langle 6.10 \rangle$ When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Quinapril Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Quinapril Hydrochloride

Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μ m. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly V' mL so that each mL contains about $1.2 \,\mu g$ of quinapril hydrochloride ($C_{25}H_{30}N_2O_5$.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 24 mg of quinapril hydrochloride for assay (separately, determine the water <2.48> in the same manner as Quinapril Hydrochloride), and dissolve in a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly 200 mL. Pipet 2 mL of this solution, add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of quinapril of both solutions.

Dissolution rate (%) with respect to the labeled amount of quinapril hydrochloride ($C_{25}H_{30}N_2O_5$.HCl)

 $= M_{\rm S} \times A_{\rm T}/A_{\rm S} \times V'/V \times 1/C \times 9/2$

- $M_{\rm S}$: Amount (mg) of quinapril hydrochloride for assay, calculated on the anhydrous basis
- C: Labeled amount (mg) of quinapril hydrochloride (C₂₅H₃₀N₂O₅.HCl) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

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

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

Mobile phase: While keeping the temperature not below 25° C, adjust to pH 2.0 of 0.1 mol/L potassium dihydrogen phosphate TS with perchloric acid. To 1000 mL of this solution add 1500 mL of acetonitrile for liquid chromatography.

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

System suitability-

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of quinapril are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of quinapril is not more than 2.0%.

Assay To 20 Quinapril Hydrochloride Tablets add 300 mL of a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1), shake vigorously to disintegrate the tablets, shake again for 10 minutes, and add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly 500 mL. Centrifuge this solution, pipet V mL of the supernatant liquid, equivalent to about 6.5 mg of quinapril hydrochloride (C₂₅H₃₀N₂O₅.HCl), add exactly 4 mL of the internal standard solution, add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of quinapril hydrochloride for assay (separately, determine the water <2.48> in the same manner as Quinapril Hydrochloride), and dissolve in a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make exactly 100 mL. Pipet 25 mL of this solution, add exactly 4 mL of the internal standard solution, add a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) to make 100 mL, and use this solution as the standard solution. Perform the test with $10 \,\mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of quinapril to that of the internal standard.

Amount (mg) of quinapril hydrochloride ($C_{25}H_{30}N_2O_5$.HCl) in 1 tablet

 $= M_{\rm S} \times Q_{\rm T}/Q_{\rm S} \times 1/V \times 25/4$

 $M_{\rm S}$: Amount (mg) of quinapril hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of phosphate buffer solution, pH 7.0 and acetonitrile for liquid chromatography (1:1) (1 in 800).

Operating conditions—

Detector: An ultraviolet spectrophotometer (wavelength: 214 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

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

Mobile phase: While keeping the temperature not below 25°C, adjust to pH 2.0 of 0.2 mol/L potassium dihydrogen phosphate TS with perchloric acid. To 1000 mL of this solution add 1000 mL of acetonitrile for liquid chromatography.

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

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, quinapril 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 $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of quinapril to that of the internal standard is not more than 1.0%.

Containers and storage Container-Tight containers.

Quinidine Sulfate Hydrate

キニジン硫酸塩水和物



 $(C_{20}H_{24}N_2O_2)_2$.H₂SO₄.2H₂O: 782.94 (9S)-6'-Methoxycinchonan-9-ol hemisulfate monohydrate [6591-63-5]

Quinidine Sulfate Hydrate, when dried, contains not less than 98.5% of quinidine sulfate $[(C_{20}H_{24}N_2O_2)_2, H_2SO_4: 746.91]$.

Description Quinidine Sulfate Hydrate occurs as white crystals. It is odorless, and has a very bitter taste.

It is freely soluble in ethanol (95) and in boiling water, sparingly soluble in water, and practically insoluble in diethyl ether. Quinidine Sulfate Hydrate, previously dried, is freely soluble in chloroform.

It darkens gradually by light.

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 0.01 g of Quinidine Sulfate Hydrate in 10 mL of water and 2 to 3 drops of dilute sulfuric acid: a blue fluorescence is produced.

(2) To 5 mL of an aqueous 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) To 5 mL of an aqueous 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 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 $\langle 1.09 \rangle$ for sulfate.

pH <2.54> 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) 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° C for 10 minutes. After cooling, filter through a tared glass filter (G4) by gentle suction. Wash the residue with five 10-mL portions of a mixture of chloroform and ethanol (99.5) (2:1), and dry at 105° C for 1 hour: the mass of the residue is not more than 2.0 mg.

(2) Related substances—Dissolve 20 mg of Quinidine Sulfate Hydrate in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of cinchonine 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 exactly $50 \,\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of the sample solution by the automatic integration method, and calculate their amount by the area percentage method: the amount of dihydroquinidine sulfate is not more than 15.0%, and those of quinine sulfate and dihydroquinine sulfate are 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 inside diameter and about 25 cm in length, packed with octadecylsilanized silica gel (10 μ m in particle diameter).

Temperature: Room temperature.

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.

Selection of column: Dissolve 0.01 g each of Quinidine Sulfate Hydrate and quinine sulfate in 5 mL of methanol, and add the mobile phase to make 50 mL. Proceed with 50 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of quinidine, quinine, dihydroquinidine and dihydroquinine in this order with a resolution between quinidine and quinine and that between quinine and dihydroquinidine being not less than 1.2, respectively.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of cinchonine obtained from $50 \,\mu\text{L}$ of the standard solution is between 5 mm and 10 mm.

Time span of measurement: About twice as long as the retention time of quinidine beginning after the solvent peak.

(3) Readily carbonizable substances $\langle 1.15 \rangle$ —Take 0.20 g of Quinidine Sulfate Hydrate and perform the test: the solution has no more color than Matching fluid M.

Loss on drying $\langle 2.41 \rangle$ Not more than 5.0% (1 g, 130 °C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 $\langle 2.50 \rangle$ 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 crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = $24.90 \text{ mg of } (C_{20}H_{24}N_2O_2)_2.H_2SO_4$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Quinine Ethyl Carbonate



 $C_{23}H_{28}N_2O_4$: 396.48 Ethyl (8*S*,9*R*)-6'-methoxycinchonan-9-yl carbonate [83-75-0]

Quinine Ethyl Carbonate contains not less than 98.5% of $C_{23}H_{28}N_2O_4$, calculated on the dehydrated basis.

Description Quinine Ethyl Carbonate occurs as white crystals. It is odorless, and tasteless at first but slowly develops a bitter taste.

It is very soluble in methanol, freely soluble in ethanol (95) and in ethanol (99.5), soluble in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

Identification (1) Determine the absorption spectrum of a solution of Quinine Ethyl Carbonate in methanol (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Quinine Ethyl Carbonate as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $\langle 2.49 \rangle$ [α]_D²⁰: $-42.2 - -44.0^{\circ}$ (0.5 g, calculated on the dehydrated basis, methanol, 50 mL, 100 mm).

Melting point <2.60> 91 – 95°C

Purity (1) Chloride—Dissolve 0.30 g of Quinine Ethyl Carbonate in 10 mL of dilute nitric acid and 20 mL of water. To 5 mL of the solution add 2 to 3 drops of silver nitrate TS: no color develops.

(2) Sulfate $\langle 1.14 \rangle$ —Dissolve 1.0 g of Quinine Ethyl Carbonate in 5 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 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 0.048%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Quinine Ethyl Carbonate 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) Related substances—Dissolve 20 mg of Quinine Ethyl Carbonate in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of quinine sulfate in the mobile phase to make exactly 100 mL. Pipet 2 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 $10 \,\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.0I \rangle$ according to the following conditions. Determine each peak area of these solutions by the automatic integration method, and calculate the amount of a main impurity in the sample solution which appears at about 1.2 times of the retention time of quinine ethyl carbonate by the area percentage method: it is not more than 10.0%. The total area of the peaks other than the principal peak and above mentioned peak from the sample solution is not larger than the peak area of quinine from the standard solution. *Operating conditions—*

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about $40\,^\circ\text{C}$.

Mobile phase: Dissolve 1.2 g of sodium 1-octanesulfonate in 1000 mL of a mixture of water and methanol (1:1), and adjust to pH 3.5 with diluted phosphoric acid (1 in 20).

Flow rate: Adjust the flow rate so that the retention time of the peak of quinine ethyl carbonate is about 20 minutes.

Selection of column: Dissolve 5 mg each of Quinine Ethyl Carbonate and quinine sulfate in the mobile phase to make 50 mL. Proceed with $10 \,\mu$ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of quinine, dihydroquinine, quinine ethyl carbonate and the main impurity of quinine ethyl carbonate in this order with the resolution between the peaks of quinine and dihydroquinine being not less than 2.7, and between the peaks of quinine and quinine ethyl carbonate being not less than 5.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of quinine obtained from $10 \,\mu\text{L}$ of the standard solution is between 5 mm and 10 mm.

Time span of measurement: About 2 times as long as the retention time of quinine ethyl carbonate.

Water <2.48> Not more than 3.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Quinine Ethyl Carbonate, dissolve in 60 mL of acetic acid (100), add 2 mL of acetic anhydride, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
=
$$19.82 \text{ mg of } C_{23}H_{28}N_2O_4$$

Containers and storage Containers—Well-closed containers.

Quinine Hydrochloride Hydrate





C₂₀H₂₄N₂O₂.HCl.2H₂O: 396.91 (8*S*,9*R*)-6'-Methoxycinchonan-9-ol monohydrochloride dihydrate [6119-47-7]

Quinine Hydrochloride Hydrate, when dried, contains not less than 98.5% of quinine hydrochloride $(C_{20}H_{24}N_2O_2.HCl: 360.88).$

Description Quinine Hydrochloride Hydrate occurs as white crystals. It is odorless, and has a very bitter taste.

It is very soluble in ethanol (99.5), freely soluble in acetic acid (100), in acetic anhydride and in ethanol (95), soluble in water, and practically insoluble in diethyl ether.

It, previously dried, is freely soluble in chloroform. It gradually changes to brown by light.

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 produced.

(2) To 5 mL of a solution of Quinine Hydrochloride Hydrate (1 in 1000) add 1 to 2 drops of bromine TS and 1 mL of ammonia TS: a green color develops.

(3) To 5 mL of a solution of Quinine Hydrochloride Hydrate (1 in 50) 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.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_{D}^{20}$: $-245 - -255^{\circ}$ (after drying, 0.5 g, 0.1 mol/L hydrochloric acid VS, 25 mL, 100 mm).

pH $\langle 2.54 \rangle$ 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 $\langle 1.14 \rangle$ —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 10-mL portions of a mixture of chloroform and ethanol (99.5) (2:1), dry at 105° C for 1 hour, and weigh: the mass 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 sample 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 exactly 50 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of the sample 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 main 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 inside diameter and about 25 cm in length, packed with octadecylsilanized silica gel (10 μ m in particle diameter).

Column temperature: Room temperature.

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.

Selection of column: Dissolve 10 mg each of Quinine Hydrochloride and quinidine sulfate in 5 mL of methanol, and add the mobile phase to make 50 mL. Proceed with $50 \,\mu$ L of this solution under the above operating conditions. Use a column giving elution of quinidine, quinine, dihydroquinidine and dihydroquinine in this order with the resolution between quinidine and quinine, and that between quinine and dihydroquinidine being not less than 1.2, respectively.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of cinchonidine from $50 \,\mu\text{L}$ of the standard solution is between 5 mm and 10 mm.

Time span of measurement: About twice as long as the retention time of quinine beginning after the solvent peak.

(5) Readily carbonizable substances $\langle 1.15 \rangle$ —Perform the test with 0.25 g of Quinine Hydrochloride Hydrate. The solution has no more color than Matching Fluid M.

Loss on drying $\langle 2.41 \rangle$ Not more than 10.0% (1 g, 105°C, 5 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 anhydride and acetic acid (100) (7:3) by warming, cool, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = $18.04 \text{ mg of } C_{20}H_{24}N_2O_2.HCl$

Containers and storage Containers-Well-closed containers.

Storage-Light-resistant.

Quinine Sulfate Hydrate

キニーネ硫酸塩水和物



 $(C_{20}H_{24}N_2O_2)_2$. H_2SO_4 . $2H_2O$: 782.94 (8S,9R)-6'-Methoxycinchonan-9-ol hemisulfate monohydrate [6119-70-6]

Quinine Sulfate Hydrate contains not less than 98.5% of quinine sulfate $[(C_{20}H_{24}N_2O_2)_2.H_2SO_4:$ 746.91], calculated on the dried basis.

Description Quinine Sulfate Hydrate occurs as white crystals or crystalline powder. It is odorless, and has a very bitter taste.

It is freely soluble in acetic acid (100), slightly soluble in water, in ethanol (95), in ethanol (99.5) and in chloroform, and practically insoluble in diethyl ether.

It gradually changes to brown by light.

Identification (1) Determine the absorption spectrum of a solution of Quinine Sulfate Hydrate (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Quinine Sulfate Hydrate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 0.4 g of Quinine Sulfate Hydrate add 20 mL of water and 1 mL of dilute hydrochloric acid: the solution responds to the Qualitative Tests $\langle 1.09 \rangle$ for sulfate.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_D^{20}$: $-235 - -245^\circ$ (after drying, 0.5 g, 0.1 mol/L hydrochloric acid VS, 25 mL, 100 mm).

pH $\langle 2.54 \rangle$ 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 $\langle 1.07 \rangle$ —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° C for 10 minutes. After cooling, filter through a tared glass filter (G4) by gentle suction. Wash the residue with five 10-mL portions of a mixture of chloroform and ethanol (99.5) (2:1), dry at 105° C for 1 hour, and weigh: the mass of the residue is not more than 2.0 mg.

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(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 sample 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 exactly $50 \,\mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area from the sample 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 main 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 inside diameter and about 25 cm in length, packed with octadecylsilanized silica gel (10 μ m in particle diameter).

Temperature: Room temperature.

Mobile phase: A mixture of water, acetonitrile, methane sulfonic 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.

Selection of column: Dissolve 0.01 g each of Quinine Sulfate Hydrate and quinidine sulfate in 5 mL of methanol, and add the mobile phase to make 50 mL. Proceed with $50 \,\mu$ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of quinidine, quinine, dihydroquinidine and dihydroquinine in this order with the resolution between quinidine and quinine and that between quinine and dihydroquinidine being not less than 1.2.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of cinchonidine obtained from $50 \,\mu\text{L}$ of the standard solution is between 5 mm and 10 mm.

Time span of measurement: About twice as long as the retention time of quinine beginning after the solvent peak.

Loss on drying <2.41> 3.0% – 5.0% (1 g, 105°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 $\langle 2.50 \rangle$ 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 crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = $24.90 \text{ mg of } (C_{20}H_{24}N_2O_2)_2.H_2SO_4$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Rabeprazole Sodium

ラベプラゾールナトリウム





Monosodium (*RS*)-2-({[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methyl}sulfinyl)-1*H*-benzoimidazolide [*117976-90-6*]

Rabeprazole Sodium contains not less than 98.0% and not more than 101.0% of $C_{18}H_{20}N_3NaO_3S$, calculated on the dried basis.

Description Rabeprazole Sodium occurs as a white to pale yellowish white powder.

It is very soluble in water, and freely soluble in ethanol (99.5).

It dissolves in 0.01 mol/L sodium hydroxide TS.

It is hygroscopic.

A solution of Rabeprazole Sodium (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Rabeprazole Sodium in 0.01 mol/L sodium hydroxide TS (1 in 100,000) as directed under Ultravioletvisible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Rabeprazole Sodium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Rabeprazole Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Rabeprazole Sodium RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve the sample, or the sample and the RS separately in ethanol (99.5), evaporate the ethanol at 40°C, dry the residues in vacuum at 55°C for 24 hours, and perform the test with the residues.

(3) A solution of Rabeprazole Sodium (1 in 10) responds to the Qualitative Tests <1.09> for sodium salt.

Purity (1) Heavy metals $\langle 1.07 \rangle$ —Proceed with 2.0 g of Rabeprazole 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) Related substances—Dissolve 50 mg of Rabeprazole Sodium in 50 mL of a mixture of methanol and 0.01 mol/L sodium hydroxide TS (3:2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and 0.01 mol/L sodium hydroxide TS (3:2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> 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 0.7 to rabeprazole from the sample solution is not larger than 4/5 times the peak area of rabeprazole from the standard solution, the area of the peak other than rabeprazole and other than the peak mentioned above from the sample solution is not larger than 1/10 times the peak area of rabeprazole from the standard solution, and the total area of the peaks other than rabeprazole from the sample solution is not larger than the peak area of rabeprazole 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.

Time span of measurement: About 3 times as long as the retention time of rabeprazole, beginning after the solvent peak.

System suitability-

Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of methanol and 0.01 mol/L sodium hydroxide TS (3:2) to make exactly 100 mL. Confirm that the peak area of rabeprazole obtained with $10 \,\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with $10 \,\mu$ L of the standard solution.

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of rabeprazole are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rabeprazole is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Loss on drying <2.41> Not more than 1.0% (1 g, in vacuum, phosphorus (V) oxide, 24 hours. Take the sample while avoiding moisture absorption.).

Assay Take the sample to be tested while avoiding moisture absorption. Weigh accurately about 0.1 g each of Rabeprazole Sodium and Rabeprazole Sodium RS (separately determine the loss on drying <2.41> under the same conditions as Rabeprazole Sodium), dissolve each in a mixture of methanol and 0.01 mol/L sodium hydroxide TS (3:2) to make exactly 25 mL. Pipet 5 mL each of these solutions, add exactly 10 mL of the internal standard solution to each, then add a mixture of methanol and 0.01 mol/L sodium hydroxide TS (3:2) to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of rabeprazole to that of the internal standard.

Amount (mg) of sodium rabeprazole ($C_{18}H_{20}N_3NaO_3S$) = $M_S \times Q_T/Q_S$

 $M_{\rm S}$: Amount (mg) of Rabeprazole Sodium RS, calculated on the dried basis

Internal standard solution—A solution of 1-amino-2-methylnaphthalene in a mixture of methanol and 0.01 mol/L sodium hydroxide TS (3:2) (1 in 250).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 290 nm).

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

Column temperature: A constant temperature of about 30° C.

Mobile phase: A mixture of methanol and 0.05 mol/L phosphate buffer solution, pH 7.0 (3:2).

Flow rate: Adjust the flow rate so that the retention time of rabeprazole is about 5 minutes.

System suitability-

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, rabeprazole and the internal standard are eluted in this order with the resolution between these peaks being not less than 4, and the symmetry factor of the peak of rabeprazole is not more than 2.0.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of rabeprazole to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Freeze-dried Inactivated Tissue Culture Rabies Vaccine

乾燥組織培養不活化狂犬病ワクチン

Freeze-dried Inactivated Tissue Culture Rabies Vaccine is a dried preparation containing inactivated rabies virus.

It conforms to the requirements of Freeze-dried Inactivated Tissue Culture Rabies Vaccine in the Minimum Requirements of Biologic Products.

Description Freeze-dried Inactivated Tissue Culture Rabies Vaccine becomes a colorless or light yellow-red clear liquid on addition of solvent.

Ranitidine Hydrochloride





C₁₃H₂₂N₄O₃S.HCl: 350.86 (1*EZ*)-*N*-{2-[({5-[(Dimethylamino)methyl]furan-2-yl}methyl)sulfanyl]ethyl}-*N'*-methyl-2-nitroethene-1,1-diamine monohydrochloride [66357-59-3]

Ranitidine Hydrochloride, when dried, contains not less than 97.5% and not more than 102.0% of

 $C_{13}H_{22}N_4O_3S.HCl.$

Description Ranitidine Hydrochloride occurs as a white to pale yellow, crystalline or fine granular powder.

It is very soluble in water, freely soluble in methanol, and slightly soluble in ethanol (99.5).

It is hygroscopic.

It is gradually colored by light.

Melting point: about 140°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Ranitidine Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ranitidine Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ranitidine Hydrochloride as directed in the paste method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Ranitidine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Ranitidine Hydrochloride (1 in 50) responds to the Qualitative Tests $\langle 1.09 \rangle$ for chloride.

pH $\langle 2.54 \rangle$ The pH of a solution obtained by dissolving 1.0 g of Ranitidine Hydrochloride in 100 mL of water is between 4.5 and 6.0.

Purity (1) Clarity and color of solution—A solution of Ranitidine Hydrochloride (1 in 10) is clear and pale yellow to light yellow.

(2) Heavy metals $\langle 1.07 \rangle$ —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 $\langle 1.11 \rangle$ —Prepare the test solution with 1.0 g of Ranitidine Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

(4) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 0.22 g of Ranitidine Hydrochloride in methanol to make exactly 10 mL, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 6 mL, 4 mL, 2 mL and 1 mL of the standard solution (1), add to each methanol to make exactly 10 mL, and use these solutions as the standard solution (2), the standard solution (3), the standard solution (4) and the standard solution (5), respectively. Separately, dissolve 12.7 mg of ranitidinediamine in methanol to make exactly 10 mL, and use this solution as the standard solution (6). Perform the test with these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 10 μ L each of the sample solution and standard solutions (1), (2), (3), (4) and (5) on a plate of silica gel for thin-layer chromatography. Separately, spot $10 \,\mu\text{L}$ of the sample solution on the plate, then spot $10 \,\mu\text{L}$ of the standard solution (6) on the spotted position of the sample solution. Immediately develop the plate with a mixture of 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. Allow the plate to stand in iodine vapor until the spot

from the standard solution (5) appears: the spot obtained from the standard solution (6) is completely separated from the principal spot from the sample solution. The spot having Rf value of about 0.7 from the sample solution is not more intense than the spot from the standard solution (1), the spots other than the principal spot and the spot of Rf value of about 0.7 from the sample solution are not more intense than the spot from the standard solution (2), and the total amount of these related substances, calculated by comparison with the spots from the standard solutions (1), (2), (3), (4) and (5), is not more than 1.0%.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.75% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 20 mg of Ranitidine Hydrochloride and Ranitidine Hydrochloride RS, previously dried, dissolve each in the mobile phase to make exactly 200 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly $10 \,\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of ranitidine.

Amount (mg) of $C_{13}H_{22}N_4O_3S.HCl = M_S \times A_T/A_S$

 $M_{\rm S}$: Amount (mg) of Ranitidine Hydrochloride RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 322 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

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

Mobile phase: A mixture of methanol and diluted 0.5 mol/L ammonium acetate TS (1 in 5) (17:3).

Flow rate: Adjust the flow rate so that the retention time of ranitidine is about 5 minutes.

System suitability—

System performance: Dissolve 20 mg of Ranitidine Hydrochloride and 5 mg of benzalphthalide in 200 mL of the mobile phase. When the procedure is run with $10 \,\mu$ L of this solution under the above operating conditions, benzalphthalide and ranitidine 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 $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ranitidine is not more than 1.0%.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

JP XVI

Rape Seed Oil

Oleum Rapae

ナタネ油

Rape Seed Oil is the fixed oil obtained from the seed of *Brassica campestris* Linné subsp. *napus* Hooker filius et Anderson var. *nippo-oleifera* Makino (*Cruciferae*).

Description Rape Seed Oil is a clear, pale yellow, slightly viscous oil. It is odorless or has a slight odor and a mild taste.

It is miscible with diethyl ether and with petroleum diethyl ether. It is slightly soluble in ethanol (95).

Specific gravity d_{25}^{25} : 0.906 – 0.920

Acid value <1.13> Not more than 0.2.

Saponification value <1.13> 169 – 195

Unsaponifiable matters <1.13> Not more than 1.5%.

Iodine value <1.13> 95 – 127

Containers and storage Containers—Tight containers.

Rebamipide

レバミピド



C₁₉H₁₅ClN₂O₄: 370.79 (2*RS*)-2-(4-Chlorobenzoylamino)-3-(2-oxo-1,2-dihydroquinolin-4-yl)propanoic acid [90098-04-7]

Rebamipide, when dried, contains not less than 99.0% and not more than 101.0% of $C_{19}H_{15}ClN_2O_4$.

Description Rebamipide occurs as a white crystalline powder, and has a bitter taste.

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

A solution of Rebamipide in N,N-dimethylformamide (1 in 20) shows no optical rotation.

Melting point: about 291°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Rebamipide in methanol (7 in 1,000,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Rebamipide as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. (3) Perform the test with Rebamipide as directed under Flame Coloration Test $\langle 1.04 \rangle$ (2): a green color appears.

Purity (1) Chloride $\langle 1.03 \rangle$ —Dissolve 0.5 g of Rebamipide in 40 mL of *N*,*N*-dimethylformamide, 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 as follows: To 0.40 mL of 0.01 mol/L hydrochloric acid VS add 40 mL of *N*,*N*-dimethylformamide, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.028%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Rebamipide 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) Rebamipide *m*-chloro isomer—Dissolve 40 mg of Rebamipide in a mixture of water, 0.05 mol/L phosphate buffer solution, pH 6.0, and methanol (7:7:6) to make 100 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add a mixture of water, 0.05 mol/L phosphate buffer solution, pH 6.0 and methanol (7:7:6) to make exactly 20 mL. Pipet 2 mL of this solution, add a mixture of water, 0.05 mol/L phosphate buffer solution, pH 6.0 and methanol (7:7:6) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly $10 \,\mu L$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak of rebamipide m-chloro isomer, having the relative retention time of about 0.95 with respect to rebamipide, from the sample solution, is not larger than 3/8 times the area of the peak of rebamipide from the standard solution.

Operating conditions-

Detector: An ultraviolet absorption photometer (wavelength: 222 nm).

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

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

Mobile phase: To 300 mL of phosphate buffer solution, pH 6.2, add 750 mL of water. To 830 mL of this solution add 170 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of rebamipide is about 20 minutes.

System suitability-

Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of water, 0.05 mol/L phosphate buffer solution, pH 6.0 and methanol (7:7:6) to make exactly 25 mL. Confirm that the peak area of rebamipide obtained from $10 \,\mu$ L of this solution is equivalent to 15 to 25% of that of rebamipide from $10 \,\mu$ L of the standard solution.

System performance: To 1 mL of the sample solution add a mixture of water, 0.05 mol/L phosphate buffer solution, pH 6.0 and methanol (7:7:6) to make 100 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of rebamipide are not less than 11,000 and not more than 1.2, respectively.

System repeatability: When the test is repeated 6 times

with $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rebamipide is not more than 2.0%.

(4) Related substances—Perform the test with exactly 10 μ L each of the sample solution and standard solution obtained in (3) as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: each area of the peaks of rebamipide o-chloro isomer and debenzoylated isomer, having the relative retention times of about 0.5 and about 0.7, respectively, with respect to rebamipide obtained from the sample solution, is not larger than 3/8 times the peak area of rebamipide from the standard solution, the area of each peak other than rebamipide and the peak mentioned above from the sample solution is not larger than 1/4 times the peak area of rebamipide from the standard solution, and the total area of the peaks other than rebamipide from the sample solution is not larger than the peak area of rebamipide from the standard solution. For the calculation, use the peak area of rebamipide o-chloro isomer, after multiplying by the response factor, 1.4.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 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 μ m in particle diameter).

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

Mobile phase: Dissolve 2.44 g of sodium 1-decanesulfonate in 1000 mL of water and to this solution add 1000 mL of methanol and 10 mL of phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of rebamipide is about 12 minutes.

Time span of measurement: About 3 times as long as the retention time of rebamipide, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of water, 0.05 mol/L phosphate buffer solution, pH 6.0, and methanol (7:7:6) to make exactly 50 mL. Confirm that the peak area of rebamipide obtained from $10 \,\mu$ L of this solution is equivalent to 7 to 13% of that of rebamipide from $10 \,\mu$ L of the standard solution.

System performance: Dissolve 20 mg of 4-chlorobenzoate in methanol to make 50 mL. To 5 mL of this solution add 5 mL of the sample solution and a mixture of water, 0.05 mol/L phosphate buffer solution, pH 6.0, and methanol (7:7:6) to make 50 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, rebamipide and 4-chlorobenzoate are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rebamipide is not more than 2.0%.

(5) Residual solvent—Being specified separately.

Loss on drying $\langle 2.41 \rangle$ Not more than 3.0% (1 g, 105°C, 2 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Rebamipide, previously dried, dissolve in 60 mL of N,N-dimethylformamide, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L potassium hydroxide VS until the color of the solution changes from pale yellow to colorless (indicator: 2 drops of phenol red TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide VS = $37.08 \text{ mg of } C_{19}H_{15}ClN_2O_4$

Containers and storage Containers-Well-closed containers.

Storage—Light-resistant.

Rebamipide Tablets

レバミピド錠

Rebamipide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of rebamipide ($C_{19}H_{15}CIN_2O_4$: 370.79).

Method of preparation Prepare as directed under Tablets, with Rebamipide.

Identification To a quantity of powdered Rebamipide Tablets, equivalent to 30 mg of Rebamipide according to the labeled amount, add 5 mL of a mixture of methanol and ammonia solution (28) (9:1), shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 30 mg of rebamipide for assay in 5 mL of a mixture of methanol and ammonia solution (28) (9:1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 5 μ L each of the sample 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, methanol and formic acid (75:25:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution has the same Rf value as the spot from the standard solution.

Uniformity of dosage units $\langle 6.02 \rangle$ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Rebamipide Tablets add 10 mL of water, shake well for 10 minutes, add exactly 10 mL of the internal standard solution, add 10 mL of N,N-dimethylformamide, shake well for 5 minutes, and add N,N-dimethylformamide to make 50 mL. Centrifuge this solution, pipet V mL of the supernatant liquid, equivalent to 3 mg of rebamipide (C₁₉H₁₅ClN₂O₄), and add 20 mL of N,N-dimethylformamide and water to make 50 mL. Filter this solution through a membrane filter with a pore size not exceeding $0.5 \,\mu\text{m}$, discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 0.1 g of rebamipide for assay, previously dried at 105°C for 2 hours, dissolve in N,N-dimethylformamide, and add exactly 10 mL of the internal standard solution and N,N-dimethylformamide to make 50 mL. Pipet 1.5 mL of this solution, add 20 mL of N,N-dimethylformamide, add water to make 50 mL, and use this solution as the standard Amount (mg) of rebamipide (C₁₉H₁₅ClN₂O₄) = $M_{\rm S} \times Q_{\rm T}/Q_{\rm S} \times 3/2V$

 $M_{\rm S}$: Amount (mg) of rebamipide for assay

Internal standard solution—A solution of acetanilide in N,N-dimethylformamide (1 in 150).

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of diluted disodium hydrogen phosphate-citric acid buffer solution, pH 6.0 (1 in 4), as the dissolution medium, the dissolution rate in 60 minutes of Rebamipide Tablets is not less than 75%.

Start the test with 1 tablet of Rebamipide Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding $0.45 \,\mu\text{m}$. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about $22 \mu g$ of rebamipide (C₁₉H₁₅ ClN₂O₄) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of rebamipide for assay, previously dried at 105 °C for 2 hours, and dissolve in N,N-dimethylformamide to make exactly 25 mL. Pipet 2 mL of this solution, add the dissolution medium to make exactly 200 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, using the dissolution medium as the blank, and determine the absorbances, A $_{\rm T}$ and $A_{\rm S}$, at 326 nm.

of rebamipide ($C_{19}H_{15}ClN_2O_4$)

 $= M_{\rm S} \times A_{\rm T}/A_{\rm S} \times V'/V \times 1/C \times 36$

 $M_{\rm S}$: Amount (mg) of rebamipide for assay

C: Labeled amount (mg) of rebamipide (C₁₉H₁₅ClN₂O₄) in 1 tablet

Assay To 10 Rebamipide Tablets add exactly V/5 mL of the internal standard solution and 50 mL of N,N-dimethylformamide, and disintegrate the tablets with the aid of ultrasonic waves. Shake this solution for 5 minutes, add N,Ndimethylformamide to make V mL so that each mL contains about 10 mg of rebamipide ($C_{19}H_{15}ClN_2O_4$). Centrifuge this solution, and to 5 mL of the supernatant liquid add N,Ndimethylformamide to make 50 mL. To 2 mL of this solution add 20 mL of N,N-dimethylformamide and water to make 50 mL. Filter, if necessary, through a membrane filter with a pore size not exceeding $0.5 \,\mu\text{m}$, and use the filtrate as the sample solution. Separately, weigh accurately about 0.1 g of rebamipide for assay, previously dried at 105°C for 2 hours, dissolve in N,N-dimethylformamide, and add exactly 2 mL of the internal standard solution and N,N-dimethylformamide to make 100 mL. To 2 mL of this solution, add 20 mL of N,N-dimethylformamide and water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of rebamipide to that of the internal standard.

Amount (mg) of rebamipide ($C_{19}H_{15}ClN_2O_4$) = $M_S \times Q_T/Q_S \times V/100$

 $M_{\rm S}$: Amount (mg) of rebamipide for assay

Internal standard solution—A solution of acetanilide in *N*,*N*-dimethylformamide (1 in 20).

Operating conditions—

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

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

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

Mobile phase: To 300 mL of phosphate buffer solution, pH 6.2, add 750 mL of water. To 830 mL of this solution add 170 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of rebamipide is about 20 minutes.

System suitability-

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the internal standard and rebamipide are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of rebamipide to that of the internal standard is not more than 1.0%.

Containers and storage Containers-Well-closed containers.

Reserpine

レセルピン



C33H40N2O9: 608.68

Methyl (3*S*,16*S*,17*R*,18*R*,20*R*)-11,17-dimethoxy-18-(3,4,5-trimethoxybenzoyloxy)yohimban-16-carboxylate [50-55-5]

Reserpine, when dried, contains not less than 96.0% of $C_{33}H_{40}N_2O_9$.

Description Reserpine occurs as white to pale yellow crystals or crystalline powder.

It is freely soluble in acetic acid (100) and in chloroform, slightly soluble in acetonitrile, very slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It is affected by light.

Identification (1) To 1 mg of Reserpine add 1 mL of vanillin-hydrochloric acid TS, and warm: a vivid red-purple color develops.

(2) Determine the absorption spectrum of a solution of Reserpine in acetonitrile (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Reserpine RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Reserpine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Reserpine RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_{\rm D}^{20}$: $-114 - 127^{\circ}$ (after drying, 0.25 g, chloroform, 25 mL, 100 mm).

Purity Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 50 mg of Reserpine in 50 mL of acetonitrile, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area from these solutions by the automatic integration method: the total area of all peaks other than reserpine peak from the sample solution is not larger than the peak area of reserpine from the standard solution.

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen 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.

Time span of measurement: About twice as long as the retention time of reserpine.

System suitability-

Test for required detection: To exactly 2 mL of the standard solution add acetonitorile to make exactly 50 mL. Confirm that the peak area of reserpine obtained from $10 \,\mu$ L of this solution is equivalent to 3 to 5% of that from $10 \,\mu$ L of the standard solution.

System performance: Dissolve 0.01 g of Reserpine and 4 mg of butyl parahydroxybenzoate in 100 mL of acetonitrile. To 5 mL of this solution add acetonitrile to make 50 mL. When the procedure is run with $20 \,\mu$ L of this solution according to the operating conditions in the Assay, reserpine and butyl parahydroxybenzoate 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 $10 \,\mu\text{L}$ 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%.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (0.2 g, in vacuum, 60°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.2% (0.2 g).

Assay Conduct this procedure 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 mL each of these solutions, add exactly 10 mL of the internal standard solution, 5 mL of acetonitrile and water to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of reserpine to that of the internal standard.

Amount (mg) of $C_{33}H_{40}N_2O_9 = M_S \times Q_T/Q_S$

 $M_{\rm S}$: Amount (mg) of Reservine RS

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 50,000).

Operating conditions-

Detector: An ultraviolet absorption photometer (wave-length: 268 nm).

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

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

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen 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 μ L of the standard solution under the above operating conditions, reserpine 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 $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of reserpine to that of the internal standard is not more than 2.0%.

Containers and Storage Containers—Well-closed containers.

Storage—Light-resistant.

Reserpine Injection

レセルピン注射液

Reserpine Injection is an aqueous solution for injection.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of reserpine ($C_{33}H_{40}N_2O_9$: 608.68).

Method of preparation Prepare as directed under Injections, with Reserpine.

Description Reserptine Injection is a clear, colorless or pale yellow liquid.

pH: 2.5 - 4.0

Identification Measure a volume of Reserpine Injection, equivalent to 1.5 mg of Reserpine according to the labeled amount, add 10 mL of diethyl ether, shake for 10 minutes, and take the aqueous layer. If necessary, add 10 mL of diethyl ether to the aqueous layer, and shake for 10 minutes to repeat the process. To the aqueous layer add water to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: it exhibits a maximum between 265 nm and 269 nm.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter $\langle 6.06 \rangle$ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of Reserpine Injection, equivalent to about 4 mg of reserpine ($C_{33}H_{40}N_2O_9$). Separately, weigh accurately about 4 mg of Reserpine RS, previously dried in vacuum at 60°C for 3 hours. Transfer them to separate separator, add 10 mL each of water and 5 mL each of ammonia TS, and extract with one 20-mL portion of chloroform, then with three 10-mL portions of chloroform with shaking vigorously. Combine the chloroform extracts, wash with two 50-mL portions of diluted hydrochloric acid (1 in 1000), and combine the washings. Then wash the chloroform extract with two 50-mL portions of a solution of sodium hydrogen carbonate (1 in 100), and combine the all washings. Extract the combined washing with two 10-mL portions of chloroform, and combine the washings with the former chloroform extract. Transfer the chloroform solution to a 100-mL volumetric flask through a pledget of absorbent cotton previously wetted with chloroform, wash with a small amount of chloroform, dilute with chloroform to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, $A_{\rm T}$ and $A_{\rm S}$, of the sample solution and the standard solution, respectively, at 295 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

> Amount (mg) of reservine $(C_{33}H_{40}N_2O_9)$ = $M_S \times A_T/A_S$

M_S: Amount (mg) of Reserpine RS

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

0.1% Reserpine Powder

Reserpine Powder

レセルピン散 0.1%

0.1% Reserpine Powder contains not less than 0.09% and not more than 0.11% of reserpine $(C_{33}H_{40}N_2O_9: 608.68)$.

Method of preparation

Reserpine	1 g
Lactose Hydrate	a sufficient quantity
	To make 1000 g

Prepare as directed under Powders, with the above ingredients.

Identification To 0.4 g of 0.1% Reserpine Powder add 20 mL of acetonitrile, shake for 30 minutes, and centrifuge. Determine the absorption spectrum of the supernatant liquid as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: it exhibits maxima between 265 nm and 269 nm, and between 294 nm and 298 nm.

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately a quantity of 0.1% Reserpine Powder, equivalent to about 0.5 mg of reserpine (C₃₃H₄₀N₂O₉), disperse in 12 mL of water, add exactly 10 mL of the internal standard solution and 10 mL of acetonitrile, and dissolve by warming at 50°C for 15 minutes, then add water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Reserpine RS, previously dried at 60°C in vacuum for 3 hours, dissolve in acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution, 5 mL of acetonitrile and water to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Reserpine.

> Amount (mg) of reserpine $(C_{33}H_{40}N_2O_9)$ = $M_S \times Q_T/Q_S \times 1/20$

 $M_{\rm S}$: Amount (mg) of Reservine RS

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 50,000).

Containers and storage Containers-Well-closed containers.

Storage—Light-resistant.

Reserpine Tablets

レセルピン錠

Reserpine Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of reserpine ($C_{33}H_{40}N_2O_9$: 608.68).

Method of preparation Prepare as directed under Tablets, with Reserpine.

Identification Take a portion of powdered Reserpine Tablets, equivalent to 0.4 mg of Reserpine according to the labeled amount, add 20 mL of acetonitrile, shake for 30 minute, and centrifuge. Determine the absorption spectrum of the supernatant liquid as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: it exhibits maxima between 265 nm and 269 nm, and between 294 nm and 298 nm.

Uniformity of dosage units $\langle 6.02 \rangle$ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

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Conduct this procedure without exposure to daylight, using light-resistant vessels. To 1 tablet of Reserpine Tablets add 2 mL of water, disintegrate by warming at 50°C for 15 minutes while shaking. After cooling, add exactly 2 mL of the internal standard solution per 0.1 mg of reserpine (C33H40N2O9), add 2 mL of acetonitrile, warm at 50°C for 15 minutes while shaking, and after cooling, add water to make 10 mL. Centrifuge the solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of Reserpine RS, previously dried at 60°C in vacuum for 3 hours, dissolve in acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution add exactly 10 mL of the internal standard solution, 5 mL of acetonitrile and water to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Reserpine.

> Amount (mg) of reserpine $(C_{33}H_{40}N_2O_9)$ = $M_S \times Q_T/Q_S \times C/10$

M_S: Amount (mg) of Reserpine RS

C: Labeled amount (mg) of reserpine in 1 tablet

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 50,000).

Dissolution $\langle 6.10 \rangle$ When the test is performed at 100 revolutions per minute according to the Paddle method, using 500 mL of a solution of polysorbate 80 (1 in 20,000) in diluted dilute acetic acid (1 in 200) as the dissolution medium, the dissolution rate in 30 minutes of Reserpine Tablets is not less than 70%.

Start the test with 1 tablet of Reserpine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, filter through a filter laminated with polyester fibers, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, dry Reserpine RS at 60°C in vacuum for 3 hours, weigh accurately an amount 100 times the labeled amount of Reserpine Tablets, dissolve in 1 mL of chloroform and 80 mL of ethanol (95), and add the dissolution medium to make exactly 200 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 250 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and standard solution, transfer to glass-stoppered brown test tubes T and S, respectively, add exactly 5 mL each of ethanol (99.5), shake well, add exactly 1 mL each of diluted vanadium (V) oxide TS (1 in 2), shake vigorously, and allow to stand for 30 minutes. Perform the test with these solutions as directed under Fluorometry <2.22>, and determine the intensity of fluorescence, $F_{\rm T}$ and $F_{\rm S}$, at the wavelength of excitation at 400 nm and at the wavelength of fluorescence at 500 nm.

Dissolution rate (%) with respect to the labeled amount of reserpine $(C_{33}H_{40}N_2O_9)$

 $= M_{\rm S} \times F_{\rm T}/F_{\rm S} \times 1/C$

 $M_{\rm S}$: Amount (mg) of Reservine RS

C: Labeled amount (mg) of reserpine $(C_{33}H_{40}N_2O_9)$ in 1 tablet

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately and powder not less than 20 Reserpine Tablets. Weigh accurately a quantity of the powder, equivalent to about 0.5 mg of reserpine ($C_{33}H_{40}N_2O_9$), add 3 mL of water, and warm at 50°C for 15 minutes while shaking. After cooling, add exactly 10 mL of the internal standard solution, 10 mL of acetonitrile and warm at 50°C for 15 minutes while shaking. After cooling, add water to make 50 mL, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of Reserpine RS, previously dried at 60°C in vacuum for 3 hours, and dissolve in acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution, 5 mL of acetonitrile and water to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Reserpine.

Amount (mg) of reservine $(C_{33}H_{40}NO_9)$ = $M_S \times Q_T/Q_S \times 1/20$

 $M_{\rm S}$: Amount (mg) of Reservine RS

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 50,000).

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Retinol Acetate

Vitamin A Acetate



C₂₂H₃₂O₂: 328.49

(2*E*,4*E*,6*E*,8*E*)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraen-1-yl acetate [*127-47-9*]

Retinol Acetate is synthetic retinol acetate or synthetic retinol acetate diluted with fixed oil.

It contains not less than 2,500,000 Vitamin A Units per gram.

A suitable antioxidant may be added.

It contains not less than 95.0% and not more than 105.0% of the labeled Units.

Description Retinol Acetate occurs as pale yellow to yellow-red crystals or an ointment-like substance, and has a faint, characteristic odor, but has no rancid odor.

It is freely soluble in petroleum ether, soluble in ethanol (95), and practically insoluble in water.

It is decomposed by air and by light.

Identification Dissolve Retinol Acetate and Retinol Acetate RS, equivalent to 15,000 Units each, in 5 mL of petroleum ether, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of cyclohexane and diethyl ether (12:1) to a

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distance of about 10 cm, and air-dry the plate. Spray evenly antimony (III) chloride TS: the principal spot obtained from the sample solution is the same in color tone and Rf value with the blue spot from the standard solution.

Purity (1) Acid value $\langle 1.13 \rangle$ —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 completely. 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 $\langle 2.50 \rangle$ 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 = $V/M \times 10$

- V: Volume (mL) of 0.01 mol/L sodium thiosulfate VS consumed
- M: Amount (g) of the sample

Assay Proceed as directed in Method 1-1 under Vitamin A Assay <2.55>.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and almost well-filled, or under Nitrogen atmosphere, and in a cold place.

Retinol Palmitate

Vitamin A Palmitate





C₃₆H₆₀O₂: 524.86 (2*E*,4*E*,6*E*,8*E*)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraen-1-yl palmitate [*79-81-2*]

Retinol Palmitate is a synthetic retinol palmitate or a synthetic retinol palmitate diluted with fixed oil.

It contains not less than 1,500,000 Vitamin A Units per gram.

A suitable antioxidant may be added.

It contains not less than 95.0% and not more than 105.0% of the labeled Units.

Description Retinol Palmitate occurs as a light yellow to yellow-red, ointment-like or an oily substance. It has a faint, characteristic odor, but has no rancid odor.

It is very soluble in petroleum ether, slightly soluble in ethanol (95), and practically insoluble in water.

It is decomposed by air and by light.

Identification Dissolve Retinol Palmitate and Retinol Palmitate RS, equivalent to 15,000 Units each, in 5 mL of petroleum ether, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of cyclohexane and diethyl 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 sample solution is the same in color tone and *R*f value with the blue spot from the standard solution.

Purity (1) Acid value $\langle 1.13 \rangle$ —Take exactly 5.0 g of Retinol Palmitate, 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 completely. 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 $\langle 2.50 \rangle$ 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 = $V/M \times 10$

V: Volume (mL) of 0.01 mol/L sodium thiosulfate VS *M*: Amount (g) of the sample

Assay Proceed as directed in Method 1-1 under the Vitamin A Assay <2.55>.

Containers and storage Containers—Tight containers. Storage—Light-resistant, and almost well-filled, or under Nitrogen atmosphere, and in a cold place.

Riboflavin

Vitamin B₂

リボフラビン



C₁₇H₂₀N₄O₆: 376.36

7,8-Dimethyl-10-[(2*S*,3*S*,4*R*)-2,3,4,5-

tetrahydroxypentyl]benzo[g]pteridine-2,4(3H,10H)-dione [83-88-5]

Riboflavin, when dried, contains not less than 98.0% of $C_{17}H_{20}N_4O_6$.

1342 Riboflavin Powder / Official Monographs

Description Riboflavin occurs as yellow to orange-yellow crystals. It has a slight odor.

It is very slightly soluble in water, practically insoluble in ethanol (95), in acetic acid (100), and in diethyl ether.

It dissolves in sodium hydroxide TS.

A saturated solution of Riboflavin is neutral.

It is decomposed by light.

Melting point: about 290°C (with decomposition).

Identification (1) A solution of Riboflavin (1 in 100,000) is light yellow-green in color and has an intense yellow-green fluorescence. The color and fluorescence of the solution disappear upon the addition of 0.02 g 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) To 10 mL of a solution of Riboflavin (1 in 100,000) 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 spectrum of a solution of Riboflavin in phosphate buffer solution, pH 7.0 (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Rivoflavin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_D^{20}$: $-128 - -142^{\circ}$ Weigh accurately about 0.1 g of dried Riboflavin, dissolve in exactly 4 mL of dilute sodium hydroxide TS, add 10 mL of freshly boiled and cooled water, add exactly 4 mL of aldehyde-free ethanol while shaking, add freshly boiled and cooled water to make exactly 20 mL, and determine the 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: To 2.0 mL of 1/60 mol/L potassium dichromate VS add water to make 1000 mL.

Loss on drying $\langle 2.41 \rangle$ Not more than 1.5% (0.5 g, 105°C, 2 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.2% (1 g).

Assay Conduct this procedure 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 sample solution. Dry Riboflavin RS at 105 °C for 2 hours, weigh accurately about 15 mg, 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 sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, using water as the blank, and determine the absorbances, A_T and A_S , at 445 nm. Add 0.02 g of sodium hydrosulfite to 5 mL of each solution, shake until decolorized, and immediately measure the absorbances, A_T and $A_{\rm s}'$, of the solutions.

Amount (mg) of
$$C_{17}H_{20}N_4O_6$$

= $M_5 \times (A_T - A_T')/(A_S - A_S')$

 $M_{\rm S}$: Amount (mg) of Riboflavin RS

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Riboflavin Powder

Vitamin B₂ Powder

リボフラビン散

Riboflavin Powder contains not less than 95.0% and not more than 115.0% of the labeled amount of riboflavin (C₁₇H₂₀N₄O₆: 376.36).

Method of preparation Prepare as directed under Granules or Powders, with Riboflavin.

Identification Shake a portion of Riboflavin Powder, equivalent to 1 mg of Riboflavin according to the labeled amount, with 100 mL of water, filter, and proceed with the filtrate as directed in the Identification (1) and (2) under Riboflavin.

Purity Rancidity—Riboflavin Powder is free from any unpleasant or rancid odor or taste.

Assay The procedure should be performed under protection from direct sunlight and in light-resistant vessels. Weigh accurately Riboflavin Powder equivalent to about 15 mg of riboflavin ($C_{17}H_{20}N_4O_6$), add 800 mL of diluted acetic acid (100) (1 in 400), and extract by warming for 30 minutes with occasional shaking. Cool, dilute with water to make exactly 1000 mL, and filter through a glass filter (G4). Use this filtrate as the sample solution, and proceed as directed in the Assay under Riboflavin.

> Amount (mg) of riboflavin (C₁₇H₂₀N₄O₆) = $M_{\rm S} \times (A_{\rm T} - A_{\rm T}')/(A_{\rm S} - A_{\rm S}')$

M_S: Amount (mg) of Riboflavin RS

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Riboflavin Butyrate

リボフラビン酪酸エステル



 $C_{33}H_{44}N_4O_{10}: 656.72$ (2R,3S,4S)-5-(7,8-Dimethyl-2,4-dioxo-3,4-dihydrobenzo[g]pteridin-10(2H)-yl)pentan-1,2,3,4-tetrayl tetrabutanoate [752-56-7]

Riboflavin Butyrate, when dried, contains not less than 98.5% of $C_{33}H_{44}N_4O_{10}$.

Description Riboflavin Butyrate occurs as orange-yellow crystals or crystalline powder. It has a slight, characteristic odor and a slightly bitter taste.

It is freely soluble in methanol, in ethanol (95) and in chloroform, slightly soluble in diethyl ether, and practically insoluble in water.

It is decomposed by light.

Identification (1) A solution of Riboflavin Butyrate in ethanol (95) (1 in 100,000) shows a light yellow-green color with a strong yellowish green fluorescence. To the solution add dilute hydrochloric acid or sodium hydroxide TS: the fluorescence disappears.

(2) Dissolve 0.01 g of Riboflavin Butyrate in 5 mL of ethanol (95), add 2 mL of a mixture of a solution of hydroxylammonium chloride (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 develops.

(3) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultravioletvisible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point <2.60> 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 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 sample solution. To 25 mL of the sample solution add water to make 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: To 25 mL of the sample solution add 1 mL of silver nitrate TS, allow to stand for 10 minutes, and filter. Wash the precipitate with four 5-mL portions 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 50 mL, add 1 mL of water, and mix (not more than 0.021%).

(2) Heavy metals $\langle 1.07 \rangle$ —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—To 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 solution 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 sample solution. Pipet 1 mL of the sample 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 these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 10 μ L each of the sample solution and 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 from the sample solution are not more intense than the spot from the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately about 40 mg of Riboflavin Butyrate, previously dried, dissolve in ethanol (95) to make exactly 500 mL, and pipet 10 mL of this solution, add ethanol (95) to make exactly 50 mL, and use this solution as the sample 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 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 sample solution and standard solution at 445 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

> Amount (mg) of $C_{33}H_{44}N_4O_{10}$ = $M_S \times A_T/A_S \times 1/2 \times 1.745$

 $M_{\rm S}$: Amount (mg) of Riboflavin RS

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Riboflavin Sodium Phosphate

Riboflavin Phosphate Vitamin B₂ Phosphate Ester

リボフラビンリン酸エステルナトリウム



 $C_{17}H_{20}N_4NaO_9P$: 478.33 Monosodium (2*R*,3*S*,4*S*)-5-(7,8-dimethyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-10(2*H*)-yl)-2,3,4trihydroxypentyl monohydrogenphosphate [*130-40-5*]

Riboflavin Sodium Phosphate contains not less than 92% of $C_{17}H_{20}N_4NaO_9P$, calculated on the anhydrous basis.

Description Riboflavin Sodium Phosphate is a yellow to orange-yellow, crystalline powder. It is odorless, and has a slightly bitter taste.

It is soluble in water, and practically insoluble in ethanol (95), in chloroform and in diethyl ether.

It is decomposed on exposure to light.

It is very hygroscopic.

Identification (1) A solution of Riboflavin Sodium Phosphate (1 in 100,000) is light yellow-green in color and has an intense yellow-green fluorescence. The color and fluorescence of the solution disappear upon the addition of 0.02 g 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) To 10 mL of a solution of Riboflavin Sodium Phosphate (1 in 100,000) 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 spectrum of a solution of Riboflavin Sodium Phosphate in phosphate buffer solution, pH 7.0, (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) To 0.05 g 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 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 $\langle 1.09 \rangle$ for sodium salt and phosphate.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_{D}^{20}$: $+38 - +43^{\circ}$ (0.3 g, calcu-

lated on the anhydrous basis, 5 mol/L hydrochloric acid TS, 20 mL, 100 mm).

pH <2.54> 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-yellow in color.

(2) 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: To 3.0 mL of 1/60 mol/L potassium dichromate VS add water to make 1000 mL.

(3) 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 sample solution. Measure exactly 5 mL each of the sample solution and Phosphoric Acid Standard Solution, transfer to separate 25-mL volumetric flasks, add 2.5 mL of hexaammonium heptamolybdate-sulfuric acid TS and 1 mL of 1-amino-2naphthol-4-sulfonic acid TS to each of these flasks, mix, and add water to make 25 mL. Allow to stand for 30 minutes at $20 \pm 1^{\circ}$ C, and perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with 5 mL of water in the same manner as a blank. Determine the absorbances, $A_{\rm T}$ and $A_{\rm S}$, of the subsequent solutions of the sample solution and standard phosphoric acid solution at 740 nm: the free phosphoric acid content is not more than 1.5%.

> Content (%) of free phosphoric acid (H₃PO₄) = $1/M \times A_T/A_S \times 258.0$

M: Amount (mg) of Riboflavin Sodium Phosphate calculated on the anhydrous basis

Water <2.48> Place 25 mL of a mixture of methanol for Karl Fischer method and ethylene glycol for Karl Fischer method (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 Conduct this procedure without exposure to daylight, using light-resistant vessels. To about 0.1 g of Riboflavin Sodium Phosphate, accurately weighed, dissolve in diluted acetic acid (100) (1 in 500) to make exactly 1000 mL, then 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 sample solution. Separately, dry Riboflavin RS at 105 °C for 2 hours, weigh accurately about 15 mg, 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 sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank, and determine the absorbances, $A_{\rm T}$ and $A_{\rm S}$, at 445 nm. Add 0.02 g of sodium hydrosulfite to 5 mL of each solution, shake until decolorized, and immediately measure the absorbances, $A_{\rm T}'$ and $A_{\rm S}'$, of the solutions.

Amount (mg) of $C_{17}H_{20}N_4NaO_9P$ = $M_S \times (A_T - A_T')/(A_S - A_S') \times 5 \times 1.271$

M_S: Amount (mg) of Riboflavin RS

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Riboflavin Sodium Phosphate Injection

Riboflavin Phosphate Injection Vitamin B₂ Phosphate Ester Injection

リボフラビンリン酸エステルナトリウム注射液

Riboflavin Sodium Phosphate Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 120.0% of the labeled amount of riboflavin ($C_{17}H_{20}N_4O_6$: 376.36).

The concentration of Riboflavin Sodium Phosphate Injection should be stated as the amount of riboflavin $(C_{17}H_{20}N_4O_6)$.

Method of preparation Prepare as directed under Injections, with Riboflavin Sodium Phosphate.

Description Riboflavin Sodium Phosphate Injection is a clear, yellow to orange-yellow liquid.

pH: 5.0 – 7.0

Identification (1) To a measured volume of Riboflavin Sodium Phosphate Injection, equivalent to 1 mg of Riboflavin according to the labeled amount, add water to make 100 mL, and proceed with this solution as directed in the Identification (1) and (2) under Riboflavin Sodium Phosphate.

(2) To a measured volume of Riboflavin Sodium Phosphate Injection, equivalent to 0.05 g of Riboflavin according to the labeled amount, and evaporate on a water bath to dryness. Proceed with this residue as directed in the Identification (4) under Riboflavin Sodium Phosphate.

Bacterial endotoxins <4.01> Less than 10 EU/mg.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter $\langle 6.06 \rangle$ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. To an accurately measured volume of Riboflavin Sodium Phosphate Injection, equivalent to about 15 mg of riboflavin ($C_{17}H_{20}N_4O_6$), add diluted acetic acid (100) (1 in 500) to make exactly 1000 mL, and use this solution as the sample solution. Proceed as directed in the Assay under Riboflavin Sodium Phosphate. Amount (mg) of Riboflavin ($C_{17}H_{20}N_4O_6$) = $M_S \times (A_T - A_T')/(A_S - A_S')$

 $M_{\rm S}$: Amount (mg) of Riboflavin RS

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.

Ribostamycin Sulfate



C17H34N4O10.xH2SO4

2,6-Diamino-2,6-dideoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-[β -D-ribofuranosyl-(1 \rightarrow 5)]-2-deoxy-D-streptamine sulfate [53797-35-6]

Ribostamycin Sulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of *Streptomyces ribosidificus*.

It contains not less than $680 \,\mu g$ (potency) and not more than $780 \,\mu g$ (potency) per mg, calculated on the dried basis. The potency of Ribostamycin Sulfate is expressed as mass (potency) of ribostamycin (C₁₇H₃₄N₄O₁₀: 454.47).

Description Ribostamycin Sulfate occurs as a white to yellowish white powder.

It is freely 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 boil: 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 sample solution and standard solution. Perform the test with these solutions as directed under Thinlayer Chromatography $\langle 2.03 \rangle$. Spot 5 μ L each of the sample 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 0.2% ninhydrin-water saturated 1-butanol TS, and heat at 100°C for 10 minutes: the principal spots obtained from the sample solution and standard solution show a purple-brown color and the same Rf 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.

Optical rotation $\langle 2.49 \rangle$ [α]_D²⁰: +42 - +49° (after drying, 0.25 g, water, 25 mL, 100 mm).

pH $\langle 2.54 \rangle$ The pH of a solution obtained by dissolving 1.0 g 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 or pale yellow.

(2) Heavy metals $\langle 1.07 \rangle$ —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 $\langle 1.11 \rangle$ —Prepare the test solution 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 exactly 20 mL, and use this solution as the sample solution. Pipet 5 mL of the sample 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 $\langle 2.03 \rangle$. Spot 5 μ L each of the sample 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 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at 100°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 5.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics $\langle 4.02 \rangle$ according to the following conditions.

(i) Test organism-Bacillus subtilis ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Ribostamycin Sulfate RS, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer solution, 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 to 15°C and use within 20 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μ g (potency) and 5 μ g (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Ribostamycin Sulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains $20 \mu g$ (potency) and $5 \mu g$ (potency), and use these solutions as the high concentration sample solution and the

low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Rice Starch

Amylum Oryzae

コメデンプン

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (\bullet, \bullet) .

Rice Starch consists of the starch granules obtained from the caryopsis of *Oryza sativa* Linné (*Gramineae*).

Description Rice Starch occurs as a white mass or powder.

It is practically insoluble in water and in ethanol (99.5).

Identification (1) Examined under a microscope $\langle 5.01 \rangle$ using a mixture of water and glycerin (1:1), Rice Starch presents polyhedral, simple grains $1 - 10 \,\mu$ m, mostly $4 - 6 \,\mu$ m, in size. These simple grains often gather in ellipsoidal, compound grains $50 - 100 \,\mu$ m in diameter. The granules have a poorly visible central hilum and there are no concentric striations. Between orthogonally orientated polarizing plates or prisms, the starch granules show a distinct black cross intersecting at the hilum.

(2) To 1 g of Rice Starch add 50 mL of water, boil for 1 minute, and allow to cool: a thin, cloudy mucilage is formed.

(3) To 1 mL of the mucilage obtained in (2) add 0.05 mL of diluted iodine TS (1 in 10): an orange-red to dark-blue color is produced which disappears on heating.

pH $\langle 2.54 \rangle$ To 5.0 g of Rice Starch add 25 mL of freshly boiled and cooled water, and mix gently for 1 minute to achieve suspension. Allow to stand for 15 minutes: the pH of the solution is 5.0 to 8.0.

Purity (1) Iron—To 1.5 g of Rice Starch add 15 mL of 2 mol/L hydrochloric acid TS, mix, filter, and use the filtrate as the test solution. To 2.0 mL of Standard Iron Solution add water to make 20 mL, and use this solution as the control solution. Put 10 mL each of the test solution and the control solution in test tubes, add 2 mL of a solution of citric acid (1 in 5) and 0.1 mL of mercapto acetic acid, and mix. Add ammonia solution (28) to these solutions until the color of a litmus paper to change from red to blue, add water to make 20 mL, and mix. Transfer 10 mL each of these solutions into test tubes, allow to stand for 5 minutes, and compare the color of these solutions against a white background: the color of the test solution is not more intense than that of the control solution (not more than 10 ppm).

(2) Oxidizing substances—To 4.0 g of Rice Starch add 50 mL of water, shake for 5 minutes, and centrifuge. To 30 mL of the supernatant liquid add 1 mL of acetic acid (100) and 0.5 to 1.0 g of potassium iodide, shake, and allow to stand for 25 to 30 minutes in the dark. Add 1 mL of starch TS, and titrate $\langle 2.50 \rangle$ with 0.002 mol/L sodium thiosulfate VS until the starch-iodine color disappears. Perform a blank determi-

- (3) Sulfur dioxide—
- (i) Apparatus Use as shown in the figure.



A: Boiling flask (500 mL) B: Funnel (100 mL) C: Condenser D: Test-tube E: Tap

(ii) Procedure Introduce 150 mL of water into the boiling flask, close the tap of the funnel, and pass carbon dioxide through the whole system at a rate of $100 \pm 5 \text{ mL}$ per minute. Pass cooling water through the condenser, and place 10 mL of hydrogen peroxide-sodium hydroxide TS in the test-tube. After 15 minutes, remove the funnel without interrupting the stream of carbon dioxide, and introduce through the opening into the flask about 25 g of Rice Starch, accurately weighed, with the aid of 100 mL of water. Apply tap grease to the outside of the connection part of the funnel, and load the funnel. Close the tap of the funnel, pour 80 mL of 2 mol/L hydrochloric acid TS into the funnel, open the tap to introduce the hydrochloric acid into the flask, and close the tap while several mL of the hydrochloric acid remains, in order to avoid losing sulfur dioxide. Place the flask in a water bath, and heat the mixture for 1 hour. Open the tap of the funnel and stop the flow of carbon dioxide and also the heating and the cooling water. Transfer the contents of the test-tube with the aid of a little water to a wide-necked conical flask. Heat on a water-bath for 15 minutes and allow to cool. Add 0.1 mL of bromophenol blue TS, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until the color changes from yellow to violet-blue lasting for at least 20 seconds. Perform a blank determination in the same manner, and make any necessary correction. Calculate the amount of sulfur dioxide by applying the following formula: it is not more than 50 ppm.

> Amount (ppm) of sulfur dioxide = $V/M \times 1000 \times 3.203$

M: Amount (g) of the sample

V: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed

•(4) Foreign matter—Under a microscope $\langle 5.01 \rangle$, Rice Starch does not contain starch granules of any other origin. It may contain a minute quantity, if any, of fragments of the tissue of the original plant.

Loss on drying <2.41> Not more than 15.0% (1 g, 130°C, 90 minutes).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.6% (1 g).

◆Containers and storage Containers—Well-closed containers.◆

Rifampicin

リファンピシン



C₄₃H₅₈N₄O₁₂: 822.94

(2*S*,12*Z*,14*E*,16*S*,17*S*,18*R*,19*R*,20*R*,21*S*,22*R*,23*S*,24*E*)-5,6,9,17,19-Pentahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-8-(4-methylpiperazin-1yliminomethyl)-1,11-dioxo-1,2-dihydro-2,7-(epoxypentadeca[1,11,13]trienimino)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*.

It contains not less than 970 μ g (potency) and not more than 1020 μ g (potency) per mg, calculated on the dried basis. The potency of Rifampicin is expressed as mass (potency) of rifampicin (C₄₃H₅₈N₄O₁₂).

Description Rifampicin occurs as orange-red to red-brown, crystals or crystalline powder.

It is slightly soluble in water, in acetonitrile, in methanol and in ethanol (95).

Identification (1) To 5 mL of a solution of Rifampicin in methanol (1 in 5000) add 0.05 mol/L phosphate buffer solution, pH 7.0 to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Rifampicin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Rifampicin as directed in the potassium bromide disk method under the Infrared Spectrophotometry $\langle 2.25 \rangle$, and

compare the spectrum with the Reference Spectrum or the spectrum of Rifampicin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals $\langle 1.07 \rangle$ —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 $\langle 1.11 \rangle$ —Prepare the test solution with 1.0 g of Rifampicin according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Perform the test immediately after preparing of the sample and standard solutions. Dissolve 0.10 g of Rifampicin in 50 mL of acetonitrile, and use this solution as the sample stock solution. Pipet 5 mL of the sample stock solution, add citric acid-phosphate-acetonitrile TS to make exactly 50 mL, and use this solution as the sample solution. Separately, pipet 1 mL of the sample stock solution, and add acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add citric acid-phosphate-acetonitrile TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak appeared at the relative retention time of about 0.7 with respect to rifampicin from the sample solution is not larger than 1.5 times the peak area of rifampicin from the standard solution, the area of the peak other than rifampicin and the peak mentioned above from the sample solution is not larger than the peak area of rifampicin from the standard solution, and the total area of the peaks other than rifampicin and the peak mentioned above from the sample solution is not larger than 3.5 times the peak area of rifampicin 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.

Time span of measurement: About 3 times as long as the retention time of rifampicin beginning after the peak of the solvent.

System suitability—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add citric acid-phosphate-acetonitrile TS to make exactly 20 mL. Confirm that the peak area of rifampicin obtained from 50 μ L of this solution is equivalent to 7 to 13% of that from 50 μ L of the standard solution.

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

System repeatability: When the test is repeated 6 times with $50 \,\mu\text{L}$ 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%.

Loss on drying $\langle 2.41 \rangle$ Not more than 2.0% (1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately an amount of Rifampicin and Rifampicin RS, equivalent to about 40 mg (potency), 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 100 mL, and use these solu-

tions as the sample solution and standard solution. Perform the test with exactly $50 \,\mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of rifampicin.

Amount [
$$\mu$$
g (potency)] of C₄₃H₅₈N₄O₁₂
= $M_{\rm S} \times A_{\rm T}/A_{\rm S} \times 1000$

M_S: Amount [mg (potency)] of Rifampicin RS

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

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

Mobile phase: Dissolve 4.2 g of citric acid monohydrate and 1.4 g of sodium perchlorate in 1000 mL of a mixture of water, acetonitrile and phosphate buffer solution, pH 3.1 (11:7:2).

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 parahydroxybenzoate in acetonitrile (1 in 5000) and citric acid-phosphate-acetonitrile TS to make 50 mL. When the procedure is run with 50 μ L of this solution under the above operating conditions, butyl parahydroxybenzoate 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 $50 \,\mu\text{L}$ 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

リファンピシンカプセル

Rifampicin Capsules contain not less than 93.0%and not more than 105.0% of rifampicin (C₄₃H₅₈N₄O₁₂: 822.94).

Method of preparation Prepare as directed under Capsules, with Rifampicin.

Identification Take out the contert of Rifampicin Capsules, mix well, and powder, if necessary. Dissolve an amount of the content, equivalent to 20 mg (potency) of Rifampicin according to the labeled amount, in methanol to make 100 mL, and filter. To 5 mL of the filtrate add 0.05 mol/L phosphate buffer solution, pH 7.0 to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: it exhibits maxima between 234 nm and 238 nm, between 252 nm and 256 nm, between 331 nm and 335 nm, and between 472 nm and 476 nm.

Purity Related substances-Perform the test quickly after

JP XVI

the sample solution and standard solution are prepared. Open the capsules of not less than 20 Rifampicin Capsules, carefully take out the content, weigh accurately, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg (potency) of Rifampicin according to the labeled amount, and dissolve in acetonitrile to make exactly 10 mL. Pipet 2 mL of this solution, add a mixture of acetonitrile and methanol (1:1) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Rifampicin RS, equivalent to about 20 mg (potency), and dissolve in acetonitrile to make exactly 10 mL. Pipet 2 mL of this solution, and add the mixture of acetonitrile and methanol (1:1) to make exactly 20 mL. Pipet 1 mL of this solution, add the mixture of acetonitrile and methanol (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the amount of the peaks of quinone substance and N-oxide substance, having the relative retention time of about 0.5 and about 1.2 with respect to rifampicin, obtained from the sample solution are not more than 4.0% and not more than 1.5%, respectively. The amount of the peak other than the peaks mentioned above is not more than 1.0%, and the total amount of these related substances is not more than 2.0%. For these calculations, use the areas of the peaks of the quinone substance and Noxide substance after multiplying by their relative response factors, 1.24 and 1.16, respectively.

> Amount (mg) of quinone substance = $M_{\rm S}/M_{\rm T} \times A_{\rm Ta}/A_{\rm S} \times 2.48$

Amount (mg) of *N*-oxide substance = $M_{\rm S}/M_{\rm T} \times A_{\rm Tb}/A_{\rm S} \times 2.32$

Each amount (mg) of related substances other than quinone and N-oxide substances = $M_{\rm S}/M_{\rm T} \times A_{\rm Ti}/A_{\rm S} \times 2$

M_S: Amount [mg (potency)] of Rifampicin RS

 $M_{\rm T}$: Amount [mg (potency)] of sample

 $A_{\rm S}$: Peak area of the standard solution

 A_{Ta} : Peak area of quinone substance

 A_{Tb} : Peak area of *N*-oxide substance

 A_{Ti} : Each peak area of related substances other than quinone and *N*-oxide substances

Operating conditions—

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

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

Column temperature: A constant temperature of about $25^{\circ}C$.

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.

Time span of measurement: About 2.5 times as long as the retention time of rifampicin.

System suitability-

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Test for required detectability: To exactly 1 mL of the standard solution add a mixture of acetonitrile and methanol (1:1) to make exactly 20 mL. Confirm that the peak area of rifampicin obtained with $20 \,\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with $20 \,\mu$ L of the standard solution.

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of rifampicin is not less than 2500 and not more than 4.0, respectively.

System repeatability: When the test is repeated 6 times with $20 \,\mu\text{L}$ 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%.

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Dissolution $\langle 6.10 \rangle$ When the test is performed at 75 revolutions per minute according to the Paddle method using a sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Rifampicin Capsules is not less than 80%.

Start the test with 1 capsule of Rifampicin Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding $0.45 \,\mu\text{m}$. 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 17 μ g (potency) of rifampicin (C₄₃H₅₈N₄O₁₂) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 17 mg (potency) of Rifampicin RS, dissolve in 5 mL of methanol, and add water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, $A_{\rm T}$ and $A_{\rm S}$, at 334 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

Dissolution rate (%) with respect to the labeled amount of rifampicin (C_{43}H_{58}N_4O_{12})

 $= M_{\rm S} \times A_{\rm T}/A_{\rm S} \times V'/V \times 1/C \times 90$

 $M_{\rm S}$: Amount [mg (potency)] of Rifampicin RS C: Labeled amount [mg (potency)] of rifampicin (C₄₃H₅₈N₄O₁₂) in 1 capsule

Assay Open the capsules of not less than 20 Rifampicin Capsules, take out the content, weigh accurately the mass of the content, and powder. Weigh accurately a portion of the powder, equivalent to about 75 mg (potency) of Rifampicin, dissolve in a mixture of acetonitrile and methanol (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 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 sample solution. Separately, weigh accurately an amount of Rifampicin RS, equivalent to about 30 mg (potency), dissolve in 20 mL of a mixture of acetonitrile and methanol (1:1), and add acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add the solution prepared by dissolving 2.1 g of citric acid monohydrate,

1350 Ringer's Solution / Official Monographs

27.6 g of disodium hydrogen phosphate dodecahydrate and 3.1 g of potassium dihydrogen phosphate in 1000 mL of the 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 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of rifampicin.

Amount [mg (potency)] of rifampicin (C₄₃H₅₈N₄O₁₂) = $M_{\rm S} \times A_{\rm T}/A_{\rm S} \times 5/2$

M_S: Amount [mg (potency)] of Rifampicin RS

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 the mixture of acetonitrile and methanol (1:1), and add acetonitrile to make 100 mL. To 5 mL of this solution add 2 mL of a solution of butyl parahydroxybenzoate in the mixture of acetonitrile and methanol (1:1) (1 in 5000), then add the 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. When the procedure is run with $50 \,\mu$ L of this solution under the above operating conditions, butyl parahydroxybenzoate 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 $50 \,\mu\text{L}$ 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.

Ringer's Solution

リンゲル液

Ringer's Solution is an aqueous solution for injection.

It contains not less than 0.53 w/v% and not more than 0.58 w/v% of chlorine [as (Cl: 35.45)], and not less than 0.030 w/v% and not more than 0.036 w/v% of calcium chloride hydrate (CaCl₂.2H₂O: 147.01).

Method of preparation

Sodium Chloride	8.6 g			
Potassium Chloride	0.3 g			
Calcium Chloride Hydrate	0.33 g			
Water for Injection or Sterile Water				
for Injection in Containers	a sufficient quantity			
	To make 1000 mL			

Prepare as directed under Injections, with the above ingredients.

No preservative may be added.

Description Ringer's Solution is a clear and colorless liquid. It has a slightly saline taste.

Identification (1) Evaporate 10 mL of Ringer's Solution to 5 mL: the solution responds to the Qualitative Tests $\langle 1.09 \rangle$ for potassium salt and calcium salt.

(2) Ringer's Solution responds to the Qualitative Tests <1.09> for sodium salt and chloride.

pH <2.54> 5.0 - 7.5

Purity (1) Heavy metals $\langle 1.07 \rangle$ —Evaporate 100 mL of Ringer's Solution to about 40 mL on a water bath. Add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Control solution: to 3.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 0.3 ppm).

(2) Arsenic $\langle 1.11 \rangle$ —Perform the test with 20 mL of Ringer's Solution as the test solution (not more than 0.1 ppm).

Bacterial endotoxins <4.01> Less than 0.50 EU/mL.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter $\langle 6.06 \rangle$ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay (1) Chlorine—To 20 mL of Ringer's Solution, accurately measured, add 30 mL of water. Titrate $\langle 2.50 \rangle$ with 0.1 mol/L silver nitrate VS while shaking vigorously (indicator: 3 drops of sodium fluorescein TS).

Each mL of 0.1 mol/L silver nitrate VS = 3.545 mg of Cl

(2) Calcium chloride Hydrate—To 50 mL of Ringer's Solution, exactly measured, add 2 mL of 8 mol/L potassium hydroxide TS and 0.05 g of NN indicator, and titrate $\langle 2.50 \rangle$ immediately with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, until the color of the solution changes from red-purple to blue.

> Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 1.470 mg of CaCl₂.2H₂O

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous infusions may be used.

Risperidone

リスペリドン



C₂₃H₂₇FN₄O₂: 410.48

3-{2-[4-(6-Fluoro-1,2-benzoisoxazol-3-yl)piperidin-1-yl]ethyl}-2methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [*106266-06-2*]

Risperidone contains not less than 98.5% and not more than 101.0% of $C_{23}H_{27}FN_4O_2$, calculated on the dried basis.

Description Risperidone occurs as a white to yellowish white crystalline powder.

It is sparingly soluble in methanol and in ethanol (99.5), very slightly soluble in 2-propanol, and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Risperidone in 2-propanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Risperidone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 169 – 173°C

Purity (1) Heavy metals $\langle 1.07 \rangle$ —Proceed with 1.0 g of Risperidone 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).

(2) Related substances—Dissolve 0.10 g of Risperidone in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly $10 \,\mu L$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than risperidone obtained from the sample solution is not larger than the peak area of risperidone from the standard solution, and the total area of the peaks other than risperidone from the sample solution is not larger than 1.5 times the peak area of risperidone from the standard solution. Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μ m in particle diameter).

Column temperature: A constant temperature of about 30° C.

Mobile phase A: A solution of ammonium acetate (1 in 200).

Mobile phase B: Methanol.

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

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 - 2	70	30
2 - 17	$70 \rightarrow 30$	$30 \rightarrow 70$
17 – 22	30	70

Flow rate: 1.5 mL per minute.

Time span of measurement: About 1.6 times as long as the retention time of risperidone.

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 with $10 \,\mu$ L of this solution is equivalent to 7 to 13% of that with $10 \,\mu$ L of the standard solution.

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of risperidone are not less than 10,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ 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%.

(3) Residual solvent—Being specified separately.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, in vacuum, 80°C, 4 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 0.16 g of Risperidone, dissolve in 70 mL of a mixture of 2-butanone and acetic acid (100) (7:1), and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = $20.52 \text{ mg of } C_{23}H_{27}FN_4O_2$

Containers and storage Containers—Tight containers.

Risperidone Fine Granules

リスペリドン細粒

Risperidone Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of risperidone ($C_{23}H_{27}FN_4O_2$: 410.48).

Method of preparation Prepare as directed under Granules, with Risperidone. **Identification** To an amount of Risperidone Fine Granules, equivalent to 2 mg of Risperidone according to the labeled amount, add 100 mL of 2-propanol, shake thoroughly, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: it exhibits maxima between 277 nm and 281 nm and between 283 nm and 287 nm.

Purity Related substances—To an amount of Risperidone Fine Granules, equivalent to 2 mg of Risperidone according to the labeled amount, add 20 mL of a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2), shake, and filter through a membrane filter with a pore size not exceeding $0.45 \,\mu\text{m}$. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 1 mL of the sample solution, add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than risperidone obtained from the sample solution is not larger than 1/2 times the peak area of risperidone from the standard solution, and the total area of the peaks other than risperidone from the sample solution is not larger than the peak area of risperidone from the standard solution. For these calculations use the area of the peaks, having the relative retention time of about 0.4 and about 1.6 to risperidone, after multiplying by their relative response factors, 1.9 and 1.5, respectively. **Operating** conditions-

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

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

System suitability-

Test for required detectability: To exactly 5 mL of the standard solution add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly 50 mL. Confirm that the peak area of risperidone obtained with $10 \,\mu$ L of this solution is equivalent to 7.5 to 12.5% of that with $10 \,\mu$ L of the standard solution.

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of risperidone are not less than 4000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risperidone is not more than 2.5%.

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Risperidone Fine Granules is not less than 75%.

Start the test with an accurately weighed amount of Risperidone Fine Granules, equivalent to about 3 mg of risperidone ($C_{23}H_{27}FN_4O_2$) according to the labeled amount, withdraw not less than 20 mL of the medium at the specified

minute after starting the test, and filter through a membrane filter with a pore size not exceeding $0.45 \,\mu\text{m}$. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add diluted hydrochloric acid (1 in 137) to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of risperidone for assay (separately determine the loss on drying <2.41> under the same conditions as Risperidone), and dissolve in methanol to make exactly 50 mL. Pipet 15 mL of this solution, add methanol to make exactly 25 mL. Pipet 2 mL of this solution, and add water to make exactly 200 mL. Pipet 3 mL of this solution, add exactly 3 mL of diluted hydrochloric acid (1 in 137), and use this solution as the standard solution. Perform the test with exactly 100 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of risperidone of both solutions.

Dissolution rate (%) with respect to the labeled amount of risperidone $(C_{23}H_{27}FN_4O_2)$

 $= M_{\rm S}/M_{\rm T} \times A_{\rm T}/A_{\rm S} \times 1/C \times 54/5$

 $M_{\rm S}$: Amount (mg) of risperidone for assay, calculated on the dried basis

 $M_{\rm T}$: Amount (g) of sample

C: Labeled amount (mg) of risperidone ($C_{23}H_{27}FN_4O_2$) in 1 g

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 237 nm).

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

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

Mobile phase: To 1000 mL of a mixture of water and acetonitrile (13:7) add 1 mL of trifluoroacetic acid, and adjust to pH 3.0 with ammonia solution (28).

Flow rate: Adjust the flow rate so that the retention time of risperidone is about 3 minutes.

System suitability-

System performance: When the procedure is run with 100 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of risperidone are not less than 3500 and not more than 2.5, respectively.

System repeatability: When the test is repeated 6 times with $100 \,\mu\text{L}$ 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%.

Particle size <6.03> It meets the requirements of Fine granules.

Assay If necessary powder Risperidone Fine Granules, and weigh accurately an amount, equivalent to about 2 mg of risperidone ($C_{23}H_{27}FN_4O_2$), add 8 mL of a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2), shake, and add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly 20 mL. Filter the solution through a membrane filter with a pore size not exceeding 0.45 μ m. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of risperidone for assay (separately determine the loss on drying $\langle 2.41 \rangle$ under the same conditions as Risperidone), and dissolve in a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly 50 mL. Pipet 10 mL of this solution, add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of risperidone from each solution.

> Amount (mg) of risperidone ($C_{23}H_{27}FN_4O_2$) = $M_S \times A_T/A_S \times 1/25$

 $M_{\rm S}$: Amount (mg) of risperidone for assay, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 275 nm).

Column: A stainless steel column 3.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography $(3.5 \,\mu\text{m} \text{ in particle diameter})$.

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

Mobile phase: To 1000 mL of a mixture of water and acetonitrile (4:1) add 1.5 mL of trifluoroacetic acid, and adjust to pH 3.0 with ammonia solution (28).

Flow rate: Adjust the flow rate so that the retention time of risperidone is about 13 minutes.

System suitability-

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of risperidone are not less than 4000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risperidone is not more than 1.0%.

Containers and storage Containers-Tight containers.

Risperidone Oral Solution

リスペリドン内服液

Risperidone Oral Solution contains not less than 95.0% and not more than 105.0% of the labeled amount of risperidone ($C_{23}H_{27}FN_4O_2$: 410.48).

Method of preparation Prepare as directed under Liquids and Solutions for Oral Administration, with Risperidone.

Description Risperidone Oral Solution occurs as a clear and colorless liquid.

Identification To a volume of Risperidone Oral Solution, equivalent to 2 mg of Risperidone according to the labeled amount, add 50 mg of sodium hydrogen carbonate and 10 mL of diethyl ether, shake, centrifuge, and evaporate the supernatant liquid to dryness in lukewarm water. Determine the absorption spectrum of a solution of the residue in 100

mL of 2-propanol as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 277 nm and 281 nm and between 283 nm and 287 nm.

pH Being specified separately.

Purity Related substances-To a volume of Risperidone Oral Solution, equivalent to 2 mg of Risperidone according to the labeled amount, add methanol to make 20 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and water (9:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly $10 \,\mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than risperidone obtained from the sample solution is not larger than 1/2 times the peak area of risperidone from the standard solution, and the total area of the peaks other than risperidone from the sample solution is not larger than the peak area of risperidone from the standard solution. For these calculations use the area of the peaks, having the relative retention time of about 0.4 and about 1.6 to risperidone, after multiplying by their relative response factors, 1.9 and 1.5, respectively.

Operating conditions—

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

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

System suitability-

Test for required detectability: To exactly 5 mL of the standard solution add a mixture of methanol and water (9:1) to make exactly 50 mL. Confirm that the peak area of risperidone obtained with $10 \,\mu$ L of this solution is equivalent to 7.5 to 12.5% of that with $10 \,\mu$ L of the standard solution.

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of risperidone are not less than 4000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risperidone is not more than 2.5%.

Microbial limit <4.05> The acceptance criteria of TAMC and TYMC are 10² CFU/mL and 10¹ CFU/mL, respectively. *Escherichia coli* is not observed.

Uniformity of dosage units <6.02> The granules in singleunit containers meet the requirement of the Mass variation test.

Assay To an exact volume of Risperidone Oral Solution, equivalent to about 2 mg of risperidone ($C_{23}H_{27}FN_4O_2$) according to the labeled amount, add methanol to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of risperidone for assay (separately determine the loss on drying $\langle 2.41 \rangle$ under the same conditions as Risperidone), and dissolve in methanol to make exactly 50 mL. Pipet 10 mL of this solution, add

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10 mL of water, then add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly $10 \,\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of risperidone from each solution.

Amount (mg) of risperidone (C₂₃H₂₇FN₄O₂)
=
$$M_5 \times A_T/A_5 \times 1/25$$

 $M_{\rm S}$: Amount (mg) of risperidone for assay, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 275 nm).

Column: A stainless steel column 3.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3.5 μ m in particle diameter).

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

Mobile phase: To 1000 mL of a mixture of water and acetonitrile (4:1) add 1.5 mL of trifluoroacetic acid, and adjust to pH 3.0 with ammonia solution (28).

Flow rate: Adjust the flow rate so that the retention time of risperidone is about 13 minutes.

System suitability-

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of risperidone are not less than 4000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risperidone is not more than 1.0%.

Containers and storage Containers—Tight containers.

Risperidone Tablets

リスペリドン錠

Risperidone Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of risperidone (C₂₃H₂₇FN₄O₂: 410.48).

Method of preparation Prepare as directed under Tablets, with Risperidone.

Identification Powder Risperidone Tablets. To a portion of the powder, equivalent to 2 mg of Risperidone according to the labeled amount, add 100 mL of 2-propanol, shake thoroughly, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: it exhibits maxima between 277 nm and 281 nm and between 283 nm and 287 nm.

Purity Related substances—Powder Risperidone Tablets. To a portion of the powder, equivalent to 2 mg of Risperidone according to the labeled amount, add 20 mL of a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2), shake, and filter through a membrane filter with a pore size not exceeding 0.45 μ m. Discard the first 1 mL of the filtrate,

and use the subsequent filtrate as the sample solution. Pipet 1 mL of the sample solution, add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly $10 \,\mu L$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than risperidone obtained from the sample solution is not larger than 1/2 times the peak area of risperidone from the standard solution, and the total area of the peaks other than risperidone from the sample solution is not larger than the peak area of risperidone from the standard solution. For these calculations use the area of the peaks, having the relative retention time of about 0.4 and about 1.6 to risperidone, after multiplying by their relative response factors, 1.9 and 1.5, respectively.

Operating conditions—

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

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

System suitability-

Test for required detectability: To exactly 5 mL of the standard solution add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly 50 mL. Confirm that the peak area of risperidone obtained with $10 \,\mu$ L of this solution is equivalent to 7.5 to 12.5% of that with $10 \,\mu$ L of the standard solution.

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of risperidone are not less than 4000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risperidone is not more than 2.5%.

Uniformity of dosage units $\langle 6.02 \rangle$ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Risperidone Tablets add 3V/5 mL of a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2), shake, and add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly V mL so that each mL contains 0.1 mg of risperidone (C₂₃H₂₇FN₄O₂). Filter this solution through a membrane filter with a pore size not exceeding 0.45 μ m, discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Then, proceed as directed in the Assay.

> Amount (mg) of risperidone $(C_{23}H_{27}FN_4O_2)$ = $M_S \times A_T/A_S \times V/500$

 $M_{\rm S}$: Amount (mg) of risperidone for assay, calculated on the dried basis

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Risperidone Tablets is not less than 75%.

Start the test with 1 tablet of Risperidone Tablets, with-

draw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding $0.45 \,\mu\text{m}$. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add diluted hydrochloric acid (1 in 137) to make exactly V'mL so that each mL contains about $0.56 \mu g$ of risperidone (C23H27FN4O2) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of risperidone for assay (separately determine the loss on drying $\langle 2.41 \rangle$ under the same conditions as Risperidone), and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add methanol to make exactly 25 mL. Pipet 2 mL of this solution, and add water to make exactly 200 mL. Pipet 3 mL of this solution, add exactly 3 mL of diluted hydrochloric acid (1 in 137), and use this solution as the standard solution. Perform the test with exactly 100 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of risperidone of both solutions.

Dissolution rate (%) with respect to the labeled amount

of risperidone (C₂₃H₂₇FN₄O₂) = $M_{\rm S} \times A_{\rm T}/A_{\rm S} \times V'/V \times 1/C \times 9/5$

 $M_{\rm S}$: Amount (mg) of risperidone for assay, calculated on the dried basis

C: Labeled amount (mg) of risperidone $(C_{23}H_{27}FN_4O_2)$ in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 237 nm).

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

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

Mobile phase: To 1000 mL of a mixture of water and acetonitrile (13:7) add 1 mL of trifluoroacetic acid, and adjust to pH 3.0 with ammonia solution (28).

Flow rate: Adjust the flow rate so that the retention time of risperidone is about 3 minutes.

System suitability—

System performance: When the procedure is run with 100 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of risperidone are not less than 3500 and not more than 2.5, respectively.

System repeatability: When the test is repeated 6 times with $100 \,\mu\text{L}$ 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%.

Assay Weigh accurately the mass of not less than 20 Risperidone Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 2 mg of risperidone $(C_{23}H_{27}FN_4O_2)$, add 8 mL of a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2), shake, and add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly 20 mL. Filter the solution through a membrane filter with a pore size not exceeding 0.45 μ m. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of risperidone for assay (separately determine the loss

on drying $\langle 2.41 \rangle$ under the same conditions as Risperidone), and dissolve in a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly 50 mL. Pipet 10 mL of this solution, add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (3:2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of risperidone from each solution.

> Amount (mg) of risperidone ($C_{23}H_{27}FN_4O_2$) = $M_S \times A_T/A_S \times 1/25$

 $M_{\rm S}$: Amount (mg) of risperidone for assay, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 275 nm).

Column: A stainless steel column 3.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3.5 μ m in particle diameter).

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

Mobile phase: To 1000 mL of a mixture of water and acetonitrile (4:1) add 1.5 mL of trifluoroacetic acid, and adjust to pH 3.0 with ammonia solution (28).

Flow rate: Adjust the flow rate so that the retention time of risperidone is about 13 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of risperidone are not less than 4000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risperidone is not more than 1.0%.

Containers and storage Containers—Tight containers.

Ritodrine Hydrochloride



C₁₇H₂₁NO₃·HCl: 323.81 (1*RS*,2*SR*)-1-(4-Hydroxyphenyl)-2-{[2-(4-hydroxyphenyl)ethyl]amino}propan-1-ol monohydrochloride [*23239-51-2*]

Ritodrine Hydrochloride, when dried, contains not less than 98.0% and not more than 102.0% of $C_{17}H_{21}NO_3$.HCl.

Description Ritodrine Hydrochloride occurs as a white crystalline powder.

It is freely soluble in water, in methanol and in ethanol

(99.5).

It dissolves in 0.01 mol/L hydrochloric acid TS.

A solution of Ritodrine Hydrochloride (1 in 10) shows no optical rotation.

It is gradually colored to a light yellow by light.

Melting point: about 196°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Ritodrine Hydrochloride (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ritodrine Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ritodrine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Ritodrine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Ritodrine Hydrochloride (1 in 50) responds to the Qualitative Tests $\langle 1.09 \rangle$ (2) for chloride.

pH $\langle 2.54 \rangle$ The pH of a solution obtained by dissolving 1.0 g of Ritodrine Hydrochloride in 50 mL of water is between 4.5 and 5.5.

Purity (1) Clarity and color of solution—A solution obtained by dissolving 1.0 g of Ritodrine Hydrochloride in 10 mL of water is clear and colorless.

(2) Heavy metals $\langle 1.07 \rangle$ —Proceed with 2.0 g of Ritodrine 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) Related substances—Dissolve 20 mg of Ritodrine Hydrochloride in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak of ritodrine threo-isomer, having the relative retention time of about 1.2 with respect to ritodrine, obtained from the sample solution is not larger than 4/5 times the peak area of ritodrine from the standard solution, the area of the peak other than ritodrine and ritodrine threo-isomer is not larger than 3/10 times the peak area of ritodrine from the standard solution, and the total area of the peaks other than ritodrine and ritodrine threo-isomer is not larger than 4 times the peak area of ritodrine from the standard solution. Operating conditions-

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

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

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

Time span of measurement: About 3 times as long as the retention time of ritodrine beginning after the solvent peak. *System suitability*—

Test for required detectability: To exactly 5 mL of the standard solution add exactly 50 mL of the mobile phase. Confirm that the peak area of ritodrine obtained with $10 \,\mu$ L of this solution is equivalent to 7 to 13% of that with $10 \,\mu$ L of the standard solution.

System performance: To 20 mg of Ritodrine Hydrochloride add 50 mL of the mobile phase and 5.6 mL of sulfuric acid, and add the mobile phase to make 100 mL. Heat a portion of this solution at about 85° C for about 2 hours, and allow to cool. Pipet 10 mL of this solution, and add exactly 10 mL of 2 mol/L sodium hydroxide TS. When the procedure is run with 10 μ L of this solution under the above operating conditions, ritodrine and the threo-isomer 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 $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ritodrine is not more than 2.0%.

Loss on drying $\langle 2.41 \rangle$ Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.2% (1 g).

Assay Weigh accurately about 30 mg each of Ritodrine Hydrochloride and Ritodrine Hydrochloride RS, previously dried, and dissolve in methanol to make exactly 50 mL. Pipet 25 mL of these solutions, add exactly 5 mL of the internal standard solution, then add water to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μ L of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of ritodrine to that of the internal standard.

Amount (mg) of $C_{17}H_{21}NO_3.HCl$ = $M_S \times Q_T/Q_S$

 $M_{\rm S}$: Amount (mg) of Ritodrine Hydrochloride RS

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (3 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

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

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

Mobile phase: Dissolve 6.6 g of diammonium hydrogen phosphate and 1.1 g of sodium 1-heptanesulfonate in 700 mL of water, and add 300 mL of methanol. Adjust to pH 3.0 with phosphoric acid.

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

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, ritodrine and the internal standard are eluted in this order with the resolution between these peaks being not less then 3.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ of the standard solution under the above operat-

ing conditions, the relative standard deviation of the ratio of the peak area of ritodrine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Ritodrine Hydrochloride Tablets

リトドリン塩酸塩錠

Ritodrine Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of ritodrine hydrochloride ($C_{17}H_{21}NO_3$.HCl: 323.81).

Method of preparation Prepare as directed under Tablets, with Ritodrine Hydrochloride.

Identification To 10 mL of the filtrate obtained in the Assay add 0.01 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: it exhibits a maximum between 272 nm and 276 nm.

Uniformity of dosage units $\langle 6.02 \rangle$ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Ritodrine Hydrochloride Tablets add 9 mL of 0.01 mol/L hydrochloric acid TS, shake until the tablet is completely disintegrated, add 0.01 mol/L hydrochloric acid TS to make exactly 10 mL, and filter through a membrane filter having pore size of 0.45 μ m. Pipet 3 mL of the filtrate, add exactly 1 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Ritodrine Hydrochloride RS, previously dried at 105°C for 2 hours, and dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 3 mL of this solution, add exactly 1 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of ritodrine to that of the internal standard.

Amount (mg) of ritodrine hydrochloride (C₁₇H₂₁NO₃.HCl) = $M_{\rm S} \times Q_{\rm T}/Q_{\rm S} \times 1/5$

 $M_{\rm S}$: Amount (mg) of Ritodrine Hydrochloride RS

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (3 in 10,000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability-

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, ritodrine 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 $10 \,\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of

the peak area of ritodrine to that of the internal standard is not more than 1.0%.

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Ritodrine Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Ritodrine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding $0.45 \,\mu\text{m}$. 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 $5.6 \mu g$ of ritodrine hydrochloride (C₁₇H₂₁NO₃.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Ritodrine Hydrochloride RS, previously dried at 105°C for 2 hours, and dissolve in 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. Perform the test with exactly 80 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of ritodrine.

Dissolution rate (%) with respect to the labeled amount of ritodrine hydrochloride (C₁₇H₂₁NO₃.HCl) = $M_{\rm S} \times A_{\rm T}/A_{\rm S} \times V'/V \times 1/C \times 18$

 $M_{\rm S}$: Amount (mg) of Ritodrine Hydrochloride RS C: Labeled amount (mg) of ritodrine hydrochloride (C₁₇H₂₁NO₃.HCl) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability-

System performance: When the procedure is run with 80 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ritodrine are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with $80 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ritodrine is not more than 1.5%.

Assay To 20 Ritodrine Hydrochloride Tablets add 150 mL of 0.01 mol/L hydrochloric acid TS, shake for 20 minutes, and add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL. Filter through a glass filter (G4), and discard the first 20 mL of the filtrate. Pipet 30 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution and 0.01 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Ritodrine Hydrochloride RS, previously dried at 105°C for 2 hours, and dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 30 mL of this solution, add exactly 5 mL of the internal standard solution and 0.01 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of ritodrine to that of the internal standard.

Amount (mg) of ritodrine hydrochloride ($C_{17}H_{21}NO_3.HCl$) = $M_S \times Q_T/Q_S \times 4$

M_S: Amount (mg) of Ritodrine Hydrochloride RS

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (3 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

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

Column temperature: A constant temperature of about $25^{\circ}C$.

Mobile phase: Dissolve 6.6 g of diammonium hydrogen phosphate and 1.1 g of sodium 1-heptanesulfonate in 700 mL of water, and add 300 mL of methanol. Adjust to pH 3.0 with phosphoric acid.

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

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, ritodrine 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 $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ritodrine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Rokitamycin

ロキタマイシン



C42H69NO15: 827.99

(3R,4S,5S,6R,8R,9R,10E,12E,15R)-5-[4-*O*-Butanoyl-2,6-dideoxy-3-*C*-methyl-3-*O*-propanoyl- α -L-*ribo*-hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino- β -D-glucopyranosyloxy]-6-formylmethyl-3,9-dihydroxy-4-methoxy-8-methylhexadeca-10,12-dien-15-olide [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*.

It contains not less than $900 \,\mu g$ (potency) and not more than $1050 \,\mu g$ (potency) per mg, calculated on the anhydrous basis. The potency of Rokitamycin is expressed as mass (potency) of rokitamycin ($C_{42}H_{69}NO_{15}$).

Description Rokitamycin occurs as a white to yellowish white powder.

It is very soluble in methanol and in chloroform, freely soluble in ethanol (99.5) and in acetonitrile, and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Rokitamycin in methanol (1 in 50,000), as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Rokitamycin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Rokitamycin as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Rokitamycin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(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 $\langle 2.21 \rangle$: 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 $\langle 1.07 \rangle$ —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 sample solution. Pipet 3 mL of the sample solution, add acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly $5 \mu L$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area 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-propionylisoleucomycin A5 having the relative retention time of about 0.86 and 3"-O-propionylleucomycin A1 having the relative retention time of about 1.36 with respect to rokitamycin obtained with the sample solution are not larger than the peak area of rokitamycin with the standard solution, the area of the peak other than rokitamycin, 3"-Opropionylleucomycin A7, 3"-O-propionylisoleucomycin A5 and 3''-O-propionylleucomycin A₁ is not larger than 23/100 times the peak area of rokitamycin with the standard solution, and the total area of the peaks other than rokitamycin is not larger than 3 times the peak area of rokitamycin with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 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.

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

System suitability-

Test for required detectability: To exactly 1 mL of the standard solution add acetonitrile to make exactly 10 mL. Confirm that the peak area of rokitamycin obtained with $5 \,\mu$ L of this solution is equivalent to 7 to 13% of that with $5 \,\mu$ L of the standard solution.

System performance: When the procedure is run with $5 \,\mu L$ of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of rokitamycin are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μ L 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%.

Water $\langle 2.48 \rangle$ Not more than 3.0% (0.2 g, volumetric titration, direct titration).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.2% (1 g).

Assay Perform the test according to the Cylinder-plate

method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism-Micrococcus luteus ATCC 9341

(ii) Culture medium—Use the medium i in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH $\langle 2.54 \rangle$ of the medium so that it will be 7.8 to 8.0 after sterilization.

(iii) Standard solutions—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 solution, 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. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 containing 0.01% of polysorbate 80 to make solutions so that each mL contains $2 \mu g$ (potency) and $0.5 \mu g$ (potency), and use these solutions as the high concentration standard solution, respectively.

(iv) Sample solutions—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 solution, pH 4.5 to make exactly 100 mL. Take exactly a suitable amount of the solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 containing 0.01% of polysorbate 80 to make solutions so that each mL contains $2 \mu g$ (potency) and 0.5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Rokitamycin Tablets

ロキタマイシン錠

Rokitamycin Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of rokitamycin (C₄₂H₆₉NO₁₅: 827.99).

Method of preparation Prepare as directed under Tablets, with Rokitamycin.

Identification Take 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 as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 230 nm and 233 nm.

Uniformity of dosage units $\langle 6.02 \rangle$ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Add 50 mL of water to 1 tablet of Rokitamycin Tablets, and disintegrate. Then add 10 mL of methanol, shake well, and add water to make exactly 100 mL. Centrifuge this solution if necessary, filter through a membrane filter with a pore size not exceeding $0.5 \,\mu$ m. Discard 5 mL of the first filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 20 μ g (potency) of Rokitamycin, and use this solution as the sample 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, $A_{\rm T}$ and $A_{\rm S}$, at 232 nm of the sample solution and standard solution using water as the blank, as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$.

Amount [mg (potency)] of rokitamycin (
$$C_{42}H_{69}NO_{15}$$
)
= $M_{\rm S} \times A_{\rm T}/A_{\rm S} \times V'/V \times 1/10$

M_S: Amount [mg (potency)] of Rokitamycin RS

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Rokitamycin Tablets is not less than 80%.

Start the test with 1 tablet of Rokitamycin Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding $0.5 \,\mu\text{m}$. Discard 10 mL of the first filtrate, pipet VmL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about $22 \mu g$ (potency) of Rokitamycin according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg (potency) of Rokitamycin RS, dissolve in 10 mL of methanol, and add water to make exactly 100 mL. Pipet 5 mL of the solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_{T} and $A_{\rm S}$, at 232 nm of the sample solution and standard solution using water as the blank, as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of rokitamycin ($C_{42}H_{69}NO_{15}$)

 $= M_{\rm S} \times A_{\rm T}/A_{\rm S} \times V'/V \times 1/C \times 90$

 $M_{\rm S}$: Amount [mg (potency)] of Rokitamycin RS

C: Labeled amount [mg (potency)] of rokitamycin $(C_{42}H_{69}NO_{15})$ in 1 tablet

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics $\langle 4.02 \rangle$ under the following conditions.

(i) Test organism, culture medium and standard solutions—Proceed as directed in the Assay under Rokitamycin.

(ii) Sample solutions—Weigh accurately not less than 20 tablets of Rokitamycin Tablets, and powder. Weigh accurately an amount of contents, equivalent to about 40 mg (potency) of Rokitamycin, add 50 mL of methanol, shake vigorously, then add 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 100 mL, and centrifuge if necessary. Measure exactly a suitable quantity of this solution, add polysorbate 80 solution, prepared by adding 0.1 mol/L phosphate buffer solution, pH 8.0 to 0.1 g of polysorbate 80 to make 1000 mL, so that each mL contains $2 \mu g$ (potency) and 0.5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Expiration date 24 months after preparation.

Roxatidine Acetate Hydrochloride

ロキサチジン酢酸エステル塩酸塩



C₁₉H₂₈N₂O₄·HCl: 384.90 (3-{3-[(Piperidin-1-yl)methyl]phenoxy}propylcarbamoyl)methyl acetate monohydrochloride [*93793-83-0*]

Roxatidine Acetate Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of $C_{19}H_{28}N_2O_4$.HCl.

Description Roxatidine Acetate Hydrochloride occurs as white, crystals or crystalline powder.

It is very soluble in water, freely soluble in acetic acid (100), and sparingly soluble in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Roxatidine Acetate Hydrochloride in ethanol (99.5) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Roxatidine Acetate Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Roxatidine Acetate Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Roxatidine Acetate Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Roxatidine Acetate Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> (2) for chloride.

pH $\langle 2.54 \rangle$ Dissolve 1.0 g of Roxatidine Acetate Hydrochloride in 20 mL of water: the pH of this solution is between 4.0 and 6.0.

Melting point <2.60> 147 – 151°C (after drying).

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Roxatidine Acetate Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals $\langle 1.07 \rangle$ —Proceed with 2.0 g of Roxatidine Acetate 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 50 mg of Roxatidine Acetate Hydrochloride in 10 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (99.5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01>
according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than roxatidine acetate obtained from sample solution is not larger than 1/5 times the peak area of roxatidine acetate from the standard solution, and the total area of the peaks other than roxatidine acetate is not larger than 1/2 times the peak area of roxatidine acetate from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with cyanopropylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about $35^{\circ}C$.

Mobile phase: A mixture of hexane, ethanol (99.5), triethylamine and acetic acid (100) (384:16:2:1).

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

Time span of measurement: About 1.5 times as long as the retention time of roxatidine acetate beginning after the solvent peak.

System suitability-

Test for required detectability: To exactly 5 mL of the standard solution add ethanol (99.5) to make exactly 10 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add ethanol (99.5) to make exactly 10 mL. Confirm that the peak area of roxatidine acetate obtained with 10 μ L of this solution is equivalent to 7 to 13% of that with 10 μ L of the solution for system suitability test.

System performance: Dissolve 50 mg of roxatidine acetate hydrochloride and 10 mg of benzoic acid in 25 mL of ethanol (99.5). When the procedure is run with 10 μ L of this solution under the above operating conditions, benzoic acid and roxatidine acetate 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 $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of roxatidine acetate is not more than 1.0%.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.3% (1 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Roxatidine Acetate Hydrochloride, previously dried, dissolve in 5 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 38.49 mg of $C_{19}H_{28}N_2O_4$.HCl

Containers and storage Containers—Tight containers.

Roxatidine Acetate Hydrochloride Extended-release Capsules

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Roxatidine Acetate Hydrochloride Extended-release Capsules contain not less than 93.0% and not more than 107.0% of the labeled amount of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4$.HCl: 384.90).

Method of preparation Prepare as directed under Capsules, with Roxatidine Acetate Hydrochloride.

Identification To 1 mL of the filtrate obtained in the Assay add ethanol (99.5) to make 20 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: it exhibits maxima between 275 nm and 278 nm, and between 282 nm and 285 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take out the contents of 1 capsule of Roxatidine Acetate Hydrochloride Extended-release Capsules, add exactly V mLof ethanol (99.5) so that each mL contains about 2.5 mg of roxatidine acetate hydrochloride (C₁₉H₂₈N₂O₄.HCl), disperse the particles with the aid of ultrasonic wave, and filter through a membrane filter with pore size of not more than 1.0 μ m. To exactly 8 mL of the filtrate add exactly 2 mL of the internal standard solution, mix, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4$.HCl) = $M_S \times Q_T/Q_S \times V/20$

 $M_{\rm S}$: Amount (mg) of Roxatidine Acetate Hydrochloride RS

Internal standard solution—A solution of benzoic acid in ethanol (99.5) (1 in 500).

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rates of a 37.5-mg capsule in 45 minutes, in 90 minutes and in 8 hours are 10 - 40%, 35 - 65%, and not less than 70\%, respectively, and of a 75-mg capsule in 60 minutes, in 90 minutes and in 8 hours are 20 - 50%, 35 - 65%, and not less than 70\%, respectively.

Start the test with 1 capsule of Roxatidine Acetate Hydrochloride Extended-release Capsules, withdraw exactly 20 mL of the medium at the specified minute after starting the test, and supply exactly 20 mL of warmed water to $37 \pm 0.5^{\circ}$ C immediately after withdrawing of the medium every time, and filter the media withdrawn through a membrane filter with a pore size not exceeding 0.45 μ m. 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 42 μ g of roxatidine acetate hydrochloride (C₁₉H₂₈N₂O₄.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 21 mg of Roxatidine Acetate Hydrochloride RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and dissolve in water to make exactly 50 mL. Pipet 2 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 100 μ L each of the sample solutions and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and determine the peak areas, $A_{T(n)}$ and A_S , of roxatidine acetate of each solution.

Dissolution rate (%) with respect to the labeled amount of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4$.HCl) on the *n*th medium withdrawing (n = 1,2,3)

$$= M_{\rm S} \times \left[\frac{A_{\rm T(n)}}{A_{\rm S}} + \sum_{i=1}^{n-1} \left(\frac{A_{\rm T(i)}}{A_{\rm S}} \times \frac{1}{45} \right) \right] \times \frac{V'}{V} \times \frac{1}{C} \times 180$$

- $M_{\rm S}$: Amount (mg) of Roxatidine Acetate Hydrochloride RS
- C: Labeled amount (mg) of roxatidine acetate hydrochloride (C₁₉H₂₈N₂O₄.HCl) in 1 capsule

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

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

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

Mobile phase: A mixture of water, acetonitrile, triethylamine and acetic acid (100) (340:60:2:1).

Flow rate: Adjust the flow rate so that the retention time of roxatidine acetate is about 5 minutes.

System suitability—

System performance: When the procedure is run with 100 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of roxatidine acetate are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with $100 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of roxatidine acetate is not more than 1.0%.

Assay Take out the contents of not less than 20 Roxatidine Acetate Hydrochloride Extended-release Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 75 mg of roxatidine acetate hydrochloride (C₁₉H₂₈N₂O₄.HCl), add exactly 30 mL of ethanol (99.5), shake, and filter through a membrane filter with pore size of not more than $1.0 \,\mu\text{m}$. To exactly 8 mL of the filtrate add exactly 2 mL of the internal standard solution, mix, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Roxatidine Acetate Hydrochloride RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and dissolve in ethanol (99.5) to make exactly 20 mL. To exactly 8 mL of this solution add exactly 2 mL of the internal standard solution, mix, and use this solution as the standard solution. Perform the test with $10 \,\mu L$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of roxatidine acetate to that of the internal standard.

Amount (mg) of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4$.HCl)

$$= M_{\rm S} \times Q_{\rm T}/Q_{\rm S} \times 3/2$$

 $M_{\rm S}$: Amount (mg) of Roxatidine Acetate Hydrochloride RS

Internal standard solution—A solution of benzoic acid in ethanol (99.5) (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 25 cm in length, packed with cyanopropylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

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

Mobile phase: A mixture of hexane, ethanol (99.5), triethylamine and acetic acid (100) (384:16:2:1).

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

System suitability-

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the internal standard and roxatidine acetate are elute in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of roxatidine acetate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Roxatidine Acetate Hydrochloride Extended-release Tablets

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Roxatidine Acetate Hydrochloride Extended-release Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4$.HCl: 384.90).

Method of preparation Prepare as directed under Tablets, with Roxatidine Acetate Hydrochloride.

Identification Powder Roxatidine Acetate Hydrochloride Extended-release Tablets. To a portion of the powder, equivalent to 37.5 mg of Roxatidine Acetate Hydrochloride according to the labeled amount, add 40 mL of ethanol (99.5), and disperse the particles for 10 minutes with the aid of ultrasonic waves with occasional shaking. After shaking thoroughly, add ethanol (99.5) to make 50 mL. Filter the solution, and to 4 mL of the filtrate add ethanol (99.5) to make 25 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: it exhibits maxima between 274 nm and 278 nm and between 281 nm and 285 nm.

Uniformity of dosage units <6.02> Perform the test accord-

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ing to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Roxatidine Acetate Hydrochloride Extended-release Tablets add 5 mL of a mixture of water, triethylamine and acetic acid (100) (340:2:1), agitate for 5 minutes with the aid of ultrasonic waves with occasional shaking, then add 7.5 mL of acetonitrile, then agitate again for 5 minutes with the aid of ultrasonic waves. Add 5 mL of a mixture of water, triethylamine and acetic acid (100) (340:2:1), agitate for 5 minutes with the aid of ultrasonic waves, shake thoroughly, add a mixture of water, triethylamine and acetic acid (100) (340:2:1) to make exactly 50 mL, centrifuge, and filter the supernatant liquid. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, equivalent to 6 mg of roxatidine acetate hydrochloride (C19H28N2O4.HCl), add exactly 3 mL of the internal standard solution, then add the mobile phase to make 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4$.HCl) = $M_S \times Q_T/Q_S \times 8/V$

 $M_{\rm S}$: Amount (mg) of Roxatidine Acetate Hydrochloride RS

Internal standard solution—A solution of sodium benzoate in the mobile phase (3 in 2000).

Dissolution Being specified separately.

Assay Weigh accurately the mass of not less than 20 Roxatidine Acetate Hydrochloride Extended-release Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 37.5 mg of roxatidine acetate hydrochloride (C19H28N2O4.HCl), add 40 mL of the mobile phase, and agitate for 10 minutes with the aid of ultrasonic waves with occasional shaking. Further shake thoroughly, add the mobile phase to make exactly 50 mL, centrifuge, and filter the supernatant liquid. Discard the first 10 mL of the filtrate, pipet 8 mL of the subsequent filtrate, add exactly 3 mL of the internal standard solution, add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 38 mg of Roxatidine Acetate Hydrochloride RS, previously dried in a desiccator (in vacuum, phosphorous (V) oxide) for 4 hours, dissolve in the mobile phase to make exactly 50 mL. Pipet 8 mL of this solution, add exactly 3 mL of the internal standard solution, then add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with $20 \,\mu L$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> under the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of roxatidine acetate to that of the internal standard.

Amount (mg) of roxatidine acetate hydrochloride (C₁₉H₂₈N₂O₄.HCl) = $M_{\rm S} \times Q_{\rm T}/Q_{\rm S}$

 $M_{\rm S}$: Amount (mg) of Roxatidine Acetate Hydrochloride RS

Internal standard solution—A solution of sodium benzoate in the mobile phase (3 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-

length: 274 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

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

Mobile phase: A mixture of water, acetonitrile, triethylamine and acetic acid (100) (340:60:2:1).

Flow rate: Adjust the flow rate so that the retention time of roxatidine acetate is about 8 minutes.

System suitability-

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the internal standard and roxatidine acetate 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 $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of roxatidine acetate to that of the internal standard is not more than 1.0%.

Containers and storage Containers-Well-closed containers.

Roxatidine Acetate Hydrochloride for Injection

注射用ロキサチジン酢酸エステル塩酸塩

Roxatidine Acetate Hydrochloride for Injection is a preparation for injection which is dissolved before use.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4$.HCl: 384.90).

Method of preparation Prepare as directed under Injections, with Roxatidine Acetate Hydrochloride.

Description It occurs as white masses or powder.

Identification To an amount of Roxatidine Acetate Hydrochloride for Injection, equivalent to 75 mg of Roxatidine Acetate Hydrochloride according to the labeled amount, add 30 mL of ethanol (99.5), shake, and filter through a membrane filter with a pore size not exceeding 0.45 μ m. To 1 mL of the filtrate add ethanol (99.5) to make 20 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: it exhibits maxima between 275 nm and 279 nm and between 282 nm and 286 nm.

pH Being specified separately.

Purity Clarity and color of solution Dissolve an amount of Roxatidine Acetate Hydrochloride for Injection, equivalent to 75 mg of Roxatidine Acetate Hydrochloride according to the labeled amount, in 20 mL of isotonic sodium chloride solution: the solution is clear and colorless.

Bacterial endotoxins <4.01> Less than 4.0 EU/mg.

Uniformity of dosage units <6.02> It meets the requirements of the Mass variation test.

Foreign insoluble matter $\langle 6.06 \rangle$ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assav Dissolve with water each content of 10 Roxatidine Acetate Hydrochloride for Injection, wash the containers with water, combine the solution of the content and washings, and add water to make exactly V mL so that each mL contains about 3.75 mg of roxatidine acetate hydrochloride $(C_{19}H_{28}N_2O_4.HCl)$. Pipet 5 mL of this solution, add water to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Roxatidine Acetate Hydrochloride RS, previously dried in a desiccator (in vacuum, phosphorous (V) oxide) for 4 hours, dissolve in water to make exactly 50 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 $10 \,\mu L$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of roxatidine acetate to that of the internal standard.

Amount (mg) of roxatidine acetate hydrochloride (C₁₉H₂₈N₂O₄.HCl) in 1 Roxatidine Acetate Hydrochloride for Injection = $M_{\rm S} \times Q_{\rm T}/Q_{\rm S} \times V/50$

 $M_{\rm S}$: Amount (mg) of Roxatidine Acetate Hydrochloride RS

Internal standard solution—Dissolve 20 mg of guanine in 10 mL of 2 mol/L hydrochloric acid TS, add 50 mL of water, then add 20 mL of a solution of sodium hydroxide (1 in 25) and water to make 100 mL. To 10 mL of this solution add water to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

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

Column temperature: A constant temperature of about $40^{\circ}C$.

Mobile phase: A mixture of water, acetonitrile, triethylamine and acetic acid (100) (340:60:2:1).

Flow rate: Adjust the flow rate so that the retention time of roxatidine acetate is about 14 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the internal standard and roxatidine acetate 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 $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of roxatidine acetate to that of the internal standard is not more than 1.0%.

Containers and storage Containers-Hermetic containers.

Roxithromycin

ロキシスロマイシン



 $\begin{array}{l} C_{41}H_{76}N_{2}O_{15}: 837.05 \\ (2R,3S,4S,5R,6R,8R,9E,10R,11R,12S,13R)-\\ 5-(3,4,6-Trideoxy-3-dimethylamino-\beta-D-xylo-\\ hexopyranosyloxy)-3-(2,6-dideoxy-3-C-methyl-3-O-methyl-\\ \alpha-L-ribo-hexopyranosyloxy)-6,11,12-trihydroxy-9-\\ (2-methoxyethoxy)methoxyimino-2,4,6,8,10,12-\\ hexamethylpentadecan-13-olide\\ [80214-83-1] \end{array}$

Roxithromycin is a derivative of erythromycin.

It contains not less than 970 μ g (potency) and not more than 1020 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Roxithromycin is expressed as mass (potency) of roxithromycin (C₄₁H₇₆N₂O₁₅).

Description Roxithromycin occurs as a white crystalline powder.

It is freely soluble in ethanol (95) and in acetone, soluble in methanol, sparingly soluble in acetonitrile, and practically insoluble in water.

Identification Determine the infrared absorption spectrum of Roxithromycin as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Roxithromycin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_{D}^{20}$: $-93 - -96^{\circ}$ (0.5 g calculated on the anhydrous basis, acetone, 50 mL, 100 mm).

Purity (1) Heavy metals $\langle 1.07 \rangle$ —Proceed with 2.0 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) Related substances—Dissolve exactly 40 mg of Roxithromycin in the mobile phase A to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve exactly 20 mg of Roxithromycin RS in the mobile phase A to make exactly 10 mL. 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 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas 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 sample solution is not larger than 2 times of the peak area of roxithromycin from the standard solution. The area of the 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 area of the peaks other than roxithromycin from the sample solution is not larger than 6 times of the peak area of roxithromycin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-length: 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 μ m in particle diameter).

Column temperature: A constant temperature of about $25^{\circ}C$.

Mobile phase A: To 200 mL of a solution of ammonium dihydrogenphosphate (17 in 100) add 510 mL of water, and adjust to pH 5.3 with 2 mol/L sodium hydroxide TS. To this solution add 315 mL of acetonitrile.

Mobile phase B: A mixture of acetonitrole and water (7:3).

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

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 - 38	100	0
38 - 39	$100 \rightarrow 90$	$0 \rightarrow 10$
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 detection: To exactly 2 mL of the standard solution add the mobile phase A to make exactly 10 mL. Confirm that the peak area of roxithromycin obtained from $20 \,\mu\text{L}$ of this solution is equivalent to 15 to 25% of that from $20 \,\mu\text{L}$ of the standard solution.

System performance: Dissolve 5 mg each of Roxithromycin RS and N-demethylroxithromycin in the mobile phase A to make 100 mL. When the procedure is run with $20 \,\mu\text{L}$ of this solution under the above operating conditions, Ndemethylroxithromycin and roxithromycin are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 5 times with $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of roxithromycin is not more than 2.0%.

Water $\langle 2.48 \rangle$ Not more than 3.0% (0.3 g, volumetric titration, direct titration).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately an amount of Roxithromycin and Roxithromycin RS, equivalent to about 20 mg (potency), and dissolve separately in the mobile phase to make exactly

10 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of roxithromycin.

Amount [
$$\mu$$
g (potency)] of C₄₁H₇₆N₂O₁₅
= $M_{\rm S} \times A_{\rm T}/A_{\rm S} \times 1000$

M_S: Amount [mg (potency)] of Roxithromycin RS

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 μ m in particle diameter).

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

Mobile phase: To 200 mL of a solution of ammonium dihydrogenphosphate (17 in 100) add 510 mL of water, and adjust to pH 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 μ L of this solution under the above operating conditions, N-demethylroxithromycin and roxithromycin are eluted in this order with the resolution between these peaks being not less than 6 and the symmetry factor of the peak of roxithromycin is not more than 1.5.

System repeatability: When, the test is repeated 6 times with $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of roxithromycin is not more than 1.0%.

Containers and storage Containers-Tight containers.

Freeze-dried Live Attenuated Rubella Vaccine

乾燥弱毒生風しんワクチン

Freeze-dried Live Attenuated Rubella Vaccine is a preparation for injection which is dissolved before use. It contains live attenuated rubella virus.

It conforms to the requirements of Freeze-dried Live Attenuated Rubella Vaccine in the Minimum Requirements for Biological Products.

Description Freeze-dried Live Attenuated Rubella Vaccine becomes a colorless, yellowish or reddish clear liquid on addition of solvent.

Saccharated Pepsin

含糖ペプシン

Saccharated Pepsin is a mixture of pepsin obtained from the gastric mucosa of hog or cattle and Lactose Hydrate, and it is an enzyme drug having a proteolytic activity.

It contains not less than 3800 units and not more than 6000 units per g.

Description Saccharated Pepsin occurs as a white powder. It has a characteristic odor, and has a slightly sweet taste.

It dissolves in water to give a slightly turbid liquid, and does not dissolve in ethanol (95) and in diethyl ether.

It is slightly hygroscopic.

Purity (1) Rancidity—Saccharated Pepsin has no unpleasant or rancid odor.

(2) Acidity—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: the solution is red in color.

Loss on drying $\langle 2.41 \rangle$ Not more than 1.0% (1 g, 80°C, 4 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.5% (1 g).

Assay (i) Substrate solution—Use the substrate solution 1 described in (2) Assay for protein digestive activity under the Digestion Test $\langle 4.03 \rangle$ after adjusting the pH to 2.0.

(ii) Sample 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.

(iii) Standard solution—Weigh accurately a suitable amount of Saccharated Pepsin RS, and dissolve in ice-cold 0.01 mol/L hydrochloric acid TS to make a solution containing about 25 units per mL.

(iv) Procedure—Proceed as directed in (2) Assay for protein digestive activity under Digestion Test $\langle 4.03 \rangle$, and determine the absorbances, $A_{\rm T}$ and $A_{\rm TB}$, of the sample solution, using trichloroacetic acid TS A as the precipitation reagent. Separately, determine the absorbances, $A_{\rm S}$ and $A_{\rm SB}$, of the standard solution in the same manner as the sample solution.

$$= U_{\rm S} \times (A_{\rm T} - A_{\rm TB})/(A_{\rm S} - A_{\rm SB}) \times 1/M$$

 $U_{\rm S}$: Units per mL of the standard solution

M: Amount (g) of Saccharated Pepsin per mL of the sample solution

Containers and storage Containers—Tight containers. Storage—Not exceeding 30°C.

Saccharin



 $C_7H_5NO_3S$: 183.18 1,2-Benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide [81-07-2]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (\bullet).

Saccharin contains not less than 99.0% and not more than 101.0% of $C_7H_5NO_3S$, calculated on the dried basis.

Description Saccharin occurs as colorless or white crystals or a white crystalline powder. It has a very sweet taste.

It is sparingly soluble in ethanol (95), and slightly soluble in water.

It dissolves in sodium hydroxide TS.

•Identification Determine the infrared absorption spectrum of Saccharin as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 226 – 230°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Saccharin in 30 mL of hot water or in 50 mL of ethanol (95): the solution is clear and colorless in each case.

•(2) Heavy metals $\langle 1.07 \rangle$ —Proceed with 2.0 g of Saccharin 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) Benzoate and salicylate—To 10 mL of a saturated solution of Saccharin in hot water add 3 drops of iron (III) chloride TS: no precipitate is formed, and no red-purple to purple color develops.

•(4) o-Toluene sulfonamide—Dissolve 10 g of Saccharin in 70 mL of sodium hydroxide TS, and extract with three 30-mL portions of ethyl acetate. Combine all the ethyl acetate extracts, wash with 30 mL of a solution of sodium chloride (1 in 4), dehydrate with 5 g of anhydrous sodium sulfate, then evaporate the solvent. To the residue add exactly 5 mL of the internal standard solution to dissolve, and use this solution as the sample solution. Separately, dissolve 0.10 g of o-toluene sulfonamide in ethyl acetate to make exactly 100 mL. Pipet 1 mL of this solution, evaporate to dryness on a water bath, dissolve the residue in exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with $1 \,\mu L$ each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak height of otoluene sulfonamide to that of the internal standard: $Q_{\rm T}$ is not more than $Q_{\rm S}$.

Internal standard solution—A solution of caffeine in ethyl acetate (1 in 500).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 1 m in length, packed with siliceous earth for gas chromatography coated 3% with diethylene glycol succinate polyester for gas chromatography (180 – 250 μ m in particle diameter).

Column temperature: A constant temperature of about 200 $^{\circ}\mathrm{C}.$

Temperature of injection port: A constant temperature of about 225° C.

Temperature of detector: A constant temperature of about 250°C.

Carrier gas: Nitrogen.

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

System suitability-

System performance: When the procedure is run with $1 \mu L$ of the standard solution under the above operating conditions, the internal standard and *o*-toluene sulfonamide 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 $1 \mu L$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak height of *o*-toluene sulfonamide to that of the internal standard is not more than 2.0%.

(5) Readily carbonizable substances $\langle 1.15 \rangle$ —Perform the test with 0.20 g of Saccharin, by warming at 48 to 50°C for 10 minutes: the color of the solution is not more intense than the matching fluid A.

Loss on drying $\langle 2.41 \rangle$ Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.2% (1 g).

Assay Weigh accurately about 0.5 g of Saccharin, dissolve in 40 mL of ethanol (95), add 40 mL of water, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction.

> Each mL of 0.1 mol/L sodium hydroxide VS = $18.32 \text{ mg of } C_7H_5NO_3S$

Containers and storage Containers-Well-closed containers.

Saccharin Sodium Hydrate

Saccharin Sodium

サッカリンナトリウム水和物



 $C_7H_4NNaO_3S.2H_2O: 241.20$ 2-Sodio-1,2-benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide dihydrate [6155-57-3]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (\bullet).

Saccharin Sodium Hydrate contains not less than 99.0% and not more than 101.0% of saccharin sodium ($C_6H_4NNaO_3S$: 205.17), calculated on the anhydrous basis.

•**Description** Saccharin Sodium Hydrate occurs as colorless crystals or a white, crystalline powder. It has an intensely sweet taste, even in 10,000 dilutions.

It is freely soluble in water and in methanol, and sparingly soluble in ethanol (95) and in acetic acid (100).

It effloresces slowly and loses about half the amount of water of crystallization in air. \bullet

Identification \bullet (1) Determine the infrared absorption spectrum of Saccharin Sodium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. \bullet

(2) A solution of Saccharin Sodium Hydrate (1 in 10) responds to the Qualitative Tests $\langle 1.09 \rangle$ for sodium salt.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Saccharin Sodium Hydrate in 1.5 mL of water or in 50 mL of ethanol (95): the solution is clear and colorless.

(2) Acidity or alkalinity—Dissolve 1.0 g of Saccharin Sodium Hydrate in 10 mL of water, and add 1 drop of phenolphthalein TS: the solution is colorless. Add 1 drop of 0.1 mol/L sodium hydroxide VS to the solution: the color changes to red.

◆(3) Heavy metals $\langle 1.07 \rangle$ —Dissolve 2.0 g of Saccharin Sodium Hydrate in 40 mL of water, add 0.7 mL of dilute hydrochloric acid, dilute with water to make 50 mL, and rub the inner wall of the vessel with a glass rod until crystallization begins. Allow the solution to stand for 1 hour after the beginning of crystallization, and then filter through dry filter paper. Reject the first 10 mL of the filtrate, and take 25 mL of the subsequent 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. To 1.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL, and use this solution as the control solution (not more than 10 ppm).

(4) Benzoate and salicylate—Dissolve 0.5 g of Saccharin

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Sodium Hydrate in 10 mL of water, add 5 drops of acetic acid (31) and 3 drops of iron (III) chloride TS: no turbidity is produced, and no red-purple to purple color develops.

•(5) o-Toluene sulfonamide—Dissolve 10 g of Saccharin Sodium Hydrate in 50 mL of water, and extract with three 30-mL portions of ethyl acetate. Combine all the ethyl acetate extracts, wash with 30 mL of a solution of sodium chloride (1 in 4), dehydrate with 5 g of anhydrous sodium sulfate, and evaporate ethyl acetate. To the residue add exactly 5 mL of the internal standard solution to dissolve, and use this solution as the sample solution. Separately, dissolve 0.10 g of o-toluene sulfonamide in ethyl acetate to make exactly 100 mL. Pipet 1 mL of this solution, evaporate on a water bath to dryness, dissolve the residue in exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with $1 \,\mu L$ each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak height of otoluene sulfonamide to that of the internal standard: $Q_{\rm T}$ is not more than $Q_{\rm S}$.

Internal standard solution—A solution of caffeine in ethyl acetate (1 in 500).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A column 3 mm in inside diameter and 1 m in length, packed with siliceous earth for gas chromatography (180 to $250 \,\mu$ m in diameter), coated with diethyleneglycol succinate polyester for gas chromatography at the ratio of 3%.

Column temperature: A constant temperature of about 200°C.

Injection port temperature: A constant temperature of about 225°C.

Detector temperature: A constant temperature of about 250°C.

Carrier gas: Nitrogen.

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

System suitability—

System performance: When the procedure is run with $1 \mu L$ of the standard solution under the above operating conditions, the internal standard and *o*-toluene sulfonamide 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 $1 \mu L$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak height of *o*-toluene sulfonamide to that of the internal standard is not more than 2.0%.

(6) Readily carbonizable substances $\langle 1.15 \rangle$ —Perform the test with 0.20 g of Saccharin Sodium Hydrate. Allow the solution to stand between 48°C and 50°C for 10 minutes: the solution has no more color than Matching Fluid A.

Water $\langle 2.48 \rangle$ Not more than 15.0% (0.1 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.15 g of Saccharin Sodium Hydrate, dissolve in 50 mL of acetic acid (100), heat slightly if necessary, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
=
$$20.52 \text{ mg of } C_7H_4NNaO_3S$$

◆Containers and storage Containers—Well-closed containers.

Salazosulfapyridine

Sulfasalazine



 $C_{18}H_{14}N_4O_5S:$ 398.39 2-Hydroxy-5-[4-(pyridin-2-ylsulfamoyl)phenylazo]benzoic acid [599-79-1]

Salazosulfapyridine, when dried, contains not less than 96.0% of $C_{18}H_{14}N_4O_5S$.

Description Salazosulfapyridine occurs as a yellow to yellow-brown, fine powder. It is odorless and tasteless.

It is sparingly soluble in pyridine, slightly soluble in ethanol (95), practically insoluble in water, in chloroform and in diethyl ether.

It dissolves in sodium hydroxide TS.

Melting point: 240 – 249°C (with decomposition).

Identification (1) Dissolve 0.1 g of Salazosulfapyridine in 20 mL of dilute sodium hydroxide TS: a red-brown color develops. This color gradually fades upon gradual addition of 0.5 g of sodium hydrosulfite with shaking. Use this solution in the following tests (2) to (4).

(2) To 1 mL of the solution obtained in (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 (1) responds to the Qualitative Tests $\langle 1.09 \rangle$ for primary aromatic amines.

(4) To 1 mL of the solution obtained in (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 develops in the chloroform layer.

(5) Determine the absorption spectrum of a solution of Salazosulfapyridine in dilute sodium hydroxide TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) Chloride $\langle 1.03 \rangle$ —Dissolve 2.0 g of Salazosulfapyridine 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 using this solution as the test solution. Prepare the control solution with 0.40 mL of

JP XVI

0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(2) Sulfate $\langle 1.14 \rangle$ —Dissolve 2.0 g of Salazosulfapyridine 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 using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(3) Heavy metals $\langle 1.07 \rangle$ —Proceed with 1.0 g of Salazosulfapyridine 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 $\langle 1.11 \rangle$ —Take 1.0 g of Salazosulfapyridine in a decomposition flask, add 20 mL of nitric acid, and heat gently until it 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 slightly yellow. After cooling, add 15 mL of a saturated solution of ammonium oxalate monohydrate, 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 color standard.

Color standard: Proceed in the same manner without Salazosulfapyridine, 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 with the test solution (not more than 10 ppm).

(5) Related substances—Dissolve 0.20 g of Salazosulfapyridine in 20 mL of pyridine, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add pyridine 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 <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with 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 sample solution are not more intense than the spot from the standard solution.

(6) Salicylic acid—To 0.10 g of Salazosulfapyridine add 15 mL of diethyl ether, and shake vigorously. Add 5 mL of dilute hydrochloric acid, shake vigorously for 3 minutes, collect the diethyl ether layer, and filter. To the water layer add 15 mL of diethyl ether, shake vigorously for 3 minutes, collect the diethyl ether layer, filter, and combine the filtrates. Wash the residue on the filter paper with a small quantity of diethyl ether, and combine the washings and the filtrate. Evaporate the diethyl 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 sample solution. Separately, weigh accurately about 10 mg of salicylic acid for assay, 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, $A_{\rm T}$ and $A_{\rm S}$, at 535 nm

of the sample solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: salicylic acid content is not more than 0.5%.

Content (%) of salicylic acid (C₇H₆O₃) = $M_{\rm S} \times A_{\rm T}/A_{\rm S} \times 1/20$

 $M_{\rm S}$: Amount (mg) of salicylic acid for assay

Loss on drying $\langle 2.41 \rangle$ Not more than 2.0% (1 g, 105°C, 4 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.2% (1 g).

Assay Weigh accurately about 20 mg of Salazosulfapyridine, previously dried, and perform the test as directed in the procedure of determination for sulfur under the Oxygen Flask Combustion Method $\langle 1.06 \rangle$, using 10 mL of diluted hydrogen peroxide (30) (1 in 40) as an absorbing liquid.

Each mL of 0.005 mol/L barium perchlorate VS = $1.992 \text{ mg of } C_{18}H_{14}N_4O_5S$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Salbutamol Sulfate





 $(C_{13}H_{21}NO_{3})_2.H_2SO_4$: 576.70 (1*RS*)-2-(1,1-Dimethylethyl)amino-1-(4-hydroxy-3-hydroxymethylphenyl)ethanol hemisulfate [*51022-70-9*]

Salbutamol Sulfate, when dried, contains not less than 98.0% of $(C_{13}H_{21}NO_3)_2.H_2SO_4$.

Description Salbutamol Sulfate occurs as a white powder.

It is freely soluble in water, slightly soluble in ethanol (95), and in acetic acid (100) and practically insoluble in diethyl ether.

A solution of Salbutamol Sulfate (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Salbutamol Sulfate in 0.1 mol/L hydrochloric acid TS (1 in 12,500) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Salbutamol Sulfate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Salbutamol Sulfate (1 in 20) responds to the Qualitative Tests <1.09> 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 $\langle 1.07 \rangle$ —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 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 sample solution. Pipet 1 mL of the sample 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 $\langle 2.03 \rangle$. Spot 5 μ L each of the sample solution and 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 airdry the plate. Leave the plate in a well-closed vessel saturated with diethylamine vapor for 5 minutes, and spray evenly 4-nitrobenzenediazonium chloride TS: the spots other than the principal spot from the sample 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 Standard Boron Solution, and transfer to a platinum crucible. Add 5 mL of potassium carbonate-sodium carbonate TS, evaporate on a water bath to dryness, and dry at 120°C for 1 hour. Ignite the residue immediately. After cooling, add 0.5 mL of water and 3 mL of curcumin TS to the residue, warm gently in a water bath for 5 minutes. After cooling, add 3 mL of acetic acid-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 sample solution and standard solution. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using ethanol (95) as the blank: the absorbance of the sample solution at 555 nm is not larger than that of the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, 100°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 $\langle 2.50 \rangle$ 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 crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = $57.67 \text{ mg of } (C_{13}H_{21}NO_3)_2.H_2SO_4$

Containers and storage Containers—Tight containers.

Salicylic Acid

サリチル酸



C₇H₆O₃: 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 $C_7H_6O_3$.

Description Salicylic Acid occurs as white crystals or crystalline powder. It has a slightly acid, followed by an acrid taste.

It is freely soluble in ethanol (95) and in acetone, and slightly soluble in water.

Identification (1) A solution of Salicylic Acid (1 in 500) responds to the Qualitative Tests $\langle 1.09 \rangle$ (1) and (3) for salicylate.

(2) Determine the absorption spectrum of a solution of Salicylic Acid in ethanol (95) (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Salicylic Acid as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 158 – 161°C

Purity (1) Chloride $\langle 1.03 \rangle$ —Dissolve 5.0 g of Salicylic Acid in 90 mL of water by heating, cool, dilute with water to 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 using 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 0.008%).

(2) Sulfate $\langle 1.14 \rangle$ —To 20 mL of the filtrate obtained in (1) 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 (not more than 0.019%).

(3) Heavy metals <1.07>—Dissolve 2.0 g of Salicylic Acid in 25 mL of acetone, add 4 mL of sodium hydroxide TS, 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 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 sample solution. Separately, dissolve exactly 10 mg of phenol, exactly 25 mg of 4-hydroxyisophthalic acid and exactly 50 mg of parahydroxybenzoic acid in the mobile phase to make exactly 100 mL. 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 exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ 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 obtained from the sample solution are not larger than the area of each respective peak from the standard solution, the area of the peak other than salicylic acid and other

than the substances mentioned above is not larger than the peak area of 4-hydroxisophthalic acid from the standard solution, and the total area of 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 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about $35^{\circ}C$.

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.

Time span of measurement: About 2 times as long as the retention time of salicylic acid, beginning after the solvent peak.

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 $10 \,\mu$ L of this solution are equivalent to 14 to 26% of the area of each respective peak from $10 \,\mu$ L 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 10 mL. When the procedure is run with $10 \,\mu$ L of this solution under the above operating conditions, parahydroxybenzoic acid, 4-hydroxyisophthalic 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 $10 \,\mu\text{L}$ 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.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (2 g, silica gel, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Salicylic Acid, previously dried, dissolve in 25 mL of neutralized ethanol, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS = $13.81 \text{ mg of } C_7H_6O_3$ Containers and storage Containers-Well-closed containers.

Salicylated Alum Powder

サリチル・ミョウバン散

Salicylated Alum Powder contains not less than 2.7% and not more than 3.3% of salicylic acid (C₇H₆O₃: 138.12).

Method of preparation

Salicylic Acid, finely powdered	30 g
Dried Aluminum Potassium Su	ılfate,
very finely powdered	640 g
Talc, very finely powdered	a sufficient quantity
	To make 1000 g

Prepare as directed under Powders, with the above ingredients.

Description Salicylated Alum Powder occurs as a white powder.

Identification (1) The colored solution obtained in the Assay has a red-purple color and exhibits an absorption maximum $\langle 2.24 \rangle$ between 520 nm and 535 nm (salicylic acid).

(2) Shake 0.3 g of Salicylated Alum Powder with 5 mL of methanol, filter, and use the filtrate as the sample solution. Separately, dissolve 0.01 g of salicylic acid in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thinlayer Chromatography $\langle 2.03 \rangle$. Spot 5 μ L each of the sample solution and standard solution on the plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (45:5:1) to a distance of about 10 cm, and airdry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution and that from the standard solution show the same Rf value. Spray evenly iron (III) chloride TS upon the plate: the spot from the standard solution and the corresponding spot from the sample solution reveal a purple color.

Assay Weigh accurately about 0.33 g of Salicylated Alum Powder, add 80 mL of ethanol (95), and shake vigorously. Dilute with ethanol (95) to make exactly 100 mL, filter, and discard the first 10 mL of the filtrate. Use the subsequent filtrate as the sample solution. Dissolve about 0.1 g of salicylic acid for assay, previously dried in a desiccator (silica gel) for 3 hours and accurately weighed, in sufficient ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, dilute with ethanol (95) to make exactly 100 mL, and use the solution as the standard solution. Pipet 10 mL each of the sample solution and standard solution into stoppered test tubes respectively, to each add exactly 5 mL of a solution of iron (III) nitrate enneahydrate (1 in 200), and dilute with hydrochloric acid-potassium chloride buffer solution, pH 2.0, to make exactly 25 mL. Determine the absorbances, $A_{\rm T}$ and $A_{\rm S}$, of both solutions at 530 nm as directed under Ultravioletvisible Spectrophotometry <2.24>, using a solution prepared in the same manner with ethanol (95), instead of the sample solution, as the blank.

Amount (mg) of salicylic acid (
$$C_7H_6O_3$$
)
= $M_S \times A_T/A_S \times 1/10$

 $M_{\rm S}$: Amount (mg) of salicylic acid for assay

Containers and storage Containers-Well-closed containers.

Salicylic Acid Adhesive Plaster

サリチル酸絆創膏

Method of preparation

Adhesive Plaster consists of a mixture of the below ingredients with carefully selected rubber, resins, zinc oxide and other substances. It has adhesive properties. It spreads evenly on a fabric.

Salicylic Acid, finely powdered	500 g
Adhesive plaster base	a sufficient quantity
	To make 1000 g

Description The surface of Salicylic Acid Adhesive Plaster is whitish in color and adheres well to the skin.

Containers and storage Containers—Well-closed containers.

Storage-Light-resistant.

Salicylic Acid Spirit

サリチル酸精

Salicylic Acid Spirit contains not less than 2.7 w/v% and not more than 3.3 w/v% of salicylic acid (C₇H₆O₃: 138.12).

Method of preparation

Salicylic Acid	30 g
Glycerin	50 mL
Ethanol	a sufficient quantity
	To make 1000 mL

Prepare as directed under Spirits, with the above ingredients.

Description Salicylic Acid Spirit is a clear, colorless liquid. Specific gravity d_{20}^{20} : about 0.86

Identification The solution obtained in the Assay has a redpurple color. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: it exhibits a maximum between 520 nm and 535 nm (salicylic acid).

Alcohol number <1.01> Not less than 8.8 (Method 2).

Assay Measure exactly 10 mL of Salicylic Acid Spirit, add 10 mL of ethanol (95) and water to make exactly 100 mL. Pipet 3 mL of this solution, and dilute with hydrochloric acid-potassium chloride buffer solution, pH 2.0, to make exactly 100 mL. Use this solution as the sample solution.

Dissolve about 0.3 g of salicylic acid for assay, previously dried in a desiccator (silica gel) for 3 hours and accurately weighed, in 10 mL of alcohol and water to make exactly 100 mL. Pipet 3 mL of this solution, dilute with hydrochloric acid-potassium chloride buffer solution, pH 2.0, to make exactly 100 mL, and use this solution as the standard solution. Pipet 10 mL each of the sample solution and standard solution, to each add exactly 5 mL of a solution of iron (III) nitrate enneahydrate (1 in 200), dilute with hydrochloric acid-potassium chloride buffer solution, pH 2.0, to exactly 25 mL. Determine the absorbances $\langle 2.24 \rangle$, $A_{\rm T}$ and $A_{\rm S}$, of both solutions at 530 nm, using a blank solution prepared in

Amount (mg) of salicylic acid ($C_7H_6O_3$) = $M_S \times A_T/A_S$

the same manner with water instead of the sample solution.

 $M_{\rm S}$: Amount (mg) of salicylic acid for assay

Containers and storage Containers—Tight containers.

Compound Salicylic Acid Spirit

複方サリチル酸精

Compound Salicylic Acid Spirit contains not less than 1.8 w/v% and not more than 2.2 w/v% of salicylic acid ($C_7H_6O_3$: 138.12), and not less than 0.43 w/v% and not more than 0.53 w/v% of phenol (C_6H_6O : 94.11).

Method of preparation

Salicylic Acid		20 g
Liquefied Phenol		5 mL
Glycerin		40 mL
Ethanol		800 mL
Water, Purified Water or Purified		
Water in Containers	a sufficient	quantity
	To make	1000 mL

Prepare as directed under Spirits, with the above ingredients.

Description Compound Salicylic Acid Spirit is a clear, colorless to light red liquid.

Specific gravity d_{20}^{20} : about 0.88

Identification (1) To 1 mL of Compound Salicylic Acid Spirit add hydrochloric acid-potassium chloride buffer solution, pH 2.0, to make 200 mL, and to 5 mL of this solution add 5 mL of a solution of iron (III) nitrate enneahydrate (1 in 200): a red-purple color is produced (salicylic acid).

(2) To 1 mL of Compound Salicylic Acid Spirit add 20 mL of water and 5 mL of dilute hydrochloric acid, and extract with 20 mL of diethyl ether. Wash the diethyl ether extract with two 5-mL portions of sodium hydrogen carbonate TS, and extract with 10 mL of dilute sodium hydroxide TS. Shake 1 mL of the extract with 1 mL of sodium nitrite TS and 1 mL of dilute hydrochloric acid, allow to stand for 10 minutes, and add 3 mL of sodium hydroxide TS: a yellow color is produced (phenol).

(3) To 0.5 mL of Compound Salicylic Acid Spirit add 5 mL of dilute hydrochloric acid, extract with 5 mL of chloroform, and use the extract as the sample solution (1). To 2

mL of Compound Salicylic Acid Spirit add 5 mL of dilute hydrochloric acid, extract with 5 mL of chrloroform, wash the extract with two 5-mL portions of sodium hydrogen carbonate TS, and use the chloroform extract as the sample solution (2). Separately, dissolve 0.01 g each of salicylic acid and phenol in 5 mL each of chloroform, and use both solutions as the standard solutions (1) and (2). Perform the test with these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 5 μ L each of the sample solutions (1) and (2) and the standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (45:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots from the sample solution (1) and standard solution (1) show the same Rf value, and the spots from the sample solution (2) and the standard solution (2) show the same Rf value. Spray evenly iron (III) chloride TS upon the plate: the spot from the standard solution (1) and the corresponding spot from the sample solution (1) reveal a purple color.

Alcohol number <1.01> Not less than 7.5 (Method 2).

Assay Measure accurately 2 mL of Compound Salicylic Acid Spirit, add exactly 5 mL of the internal standard solution and diluted methanol (1 in 2) to make 100 mL, and use this solution as the sample solution. Weigh accurately about 0.2 g of salicylic acid for assay, previously dried in a desiccator (silica gel) for 3 hours, and about 50 mg of phenol for assay, dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 5 mL of the internal standard solution and diluted methanol (1 in 2) to make 100 mL, and use this solution as the standard solution. Perform the test with 15 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_{Ta} and Q_{Tb} , of the peak area of salicylic acid and phenol to that of the internal standard in the sample solution, and the ratios, Q_{Sa} and Q_{Sb} , of the peak area of salicylic acid and phenol to that of the internal standard in the standard solution.

> Amount (mg) of salicylic acid (C₇H₆O₃) = $M_{\text{Sa}} \times Q_{\text{Ta}}/Q_{\text{Sa}} \times 1/5$ Amount (mg) of phenol (C₆H₆O) = $M_{\text{Sb}} \times Q_{\text{Tb}}/Q_{\text{Sb}} \times 1/5$

 $M_{\rm Sa}$: Amount (mg) of salicylic acid for assay $M_{\rm Sb}$: Amount (mg) of phenol for assay

Internal standard solution—A solution of theophylline in methanol (1 in 1250).

Operating conditions-

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

Column: A stainless steel column about 4 mm in inside diameter and 25 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography, 5 μ m in particle diameter.

Column temperature: Room temperature.

Mobile phase: A mixture of 0.1 mol/L phosphate buffer solution, pH 7.0, and methanol (3:1).

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

Selection of column: Dissolve 0.2 g of benzoic acid, 0.2 g of salicylic acid and 0.05 g of theophylline in 100 mL of diluted methanol (1 in 2). To 10 mL of this solution add 90 mL of diluted methanol (1 in 2). Proceed with $10 \,\mu$ L of this solution under the above operating conditions. Use a column giving elution of benzoic acid, salicylic acid and theophylline in this order, and clearly dividing each peak.

Containers and storage Containers—Tight containers.

Santonin

サントニン



 $C_{15}H_{18}O_3$: 246.30 (3*S*,3a*S*,5a*S*,9b*S*)-3,5a,9-Trimethyl-3a,5,5a,9btetrahydronaphtho[1,2-*b*]furan-2,8(3*H*,4*H*)-dione [481-06-1]

Santonin, when dried, contains not less than 98.5% and not more than 101.0% of $C_{15}H_{18}O_3$.

Description Santonin occurs as colorless crystals, or a white, crystalline powder.

It is freely soluble in chloroform, sparingly soluble in ethanol (95), and practically insoluble in water.

It becomes yellow by light.

Identification (1) Determine the absorption spectrum of a solution of Santonin in ethanol (95) (3 in 250,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Santonin as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $\langle 2.49 \rangle$ [α]_D²⁰: $-170 - -175^{\circ}$ (0.2 g, chloroform, 10 mL, 100 mm).

Melting point <2.60> 172 – 175°C

Purity (1) Heavy metals $\langle 1.07 \rangle$ —Proceed with 1.0 g of Santonin 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) Alkaloids—Boil 0.5 g of Santonin with 20 mL of diluted sulfuric acid (1 in 100), cool, and filter. Dilute 10 mL of the filtrate with water to 30 mL, add 3 drops of iodine TS, and allow to stand for 3 hours: no turbidity is produced.

(3) Artemisin—Dissolve 1.0 g of powdered Santonin in 2 mL of chloroform by slight warming: the solution is clear and colorless, or any yellow color produced is not darker than Matching Fluid A.

(4) Phenols—Boil 0.20 g of Santonin with 10 mL of water, cool, and filter. To the filtrate add bromine TS until

the color of the solution becomes yellow: no turbidity is produced.

(5) Acid-coloring substances—Moisten 10 mg of Santonin with nitric acid: no color develops immediately. Moisten Santonin with sulfuric acid, previously cooled to 0° C: no color is produced immediately.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.2% (1 g).

Assay Weigh accurately about 0.25 g of Santonin, previously dried, dissolve in 10 mL of ethanol (95) by warming, add exactly 20 mL of 0.1 mol/L sodium hydroxide VS, and heat on a water bath under a reflux condenser for 5 minutes. Cool quickly, and titrate $\langle 2.50 \rangle$ the excess sodium hydroxide with 0.05 mol/L hydrochloric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium hydroxide VS = 24.63 mg of C₁₅H₁₈O₃

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Sarpogrelate Hydrochloride

サルポグレラート塩酸塩



C24H31NO6.HCl: 465.97

(2RS)-1-Dimethylamino-3-{2-[2-(3-

methoxyphenyl)ethyl]phenoxy}propan-2-yl hydrogen succinate monohydrochloride

[135159-51-2]

Sarpogrelate Hydrochloride contains not less than 98.5% and not more than 101.0% of $C_{24}H_{31}NO_6$.HCl, calculated on the anhydrous basis.

Description Sarpogrelate Hydrochloride occurs as a white crystalline powder.

It is slightly soluble in water and in ethanol (99.5).

It dissolves in 0.01 mol/L hydrochloric acid TS.

A solution of Sarpogrelate Hydrochloride (1 in 100) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Sarpogrelate Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Sarpogrelate Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Sarpogrelate Hydrochloride as directed in the potassium chlo-

ride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Sarpogrelate Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize the sample and the RS separately with acetone by heating and suspending, filter and dry the crystals, and perform the test with the crystals.

(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. This solution responds to the Qualitative Tests $\langle 1.09 \rangle$ for chloride.

Purity (1) Heavy metals $\langle 1.07 \rangle$ —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 $\langle 1.11 \rangle$ —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 the determination within 3 hours after preparing the sample solution. Dissolve 20 mg of Sarpogrelate Hydrochloride in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly $10 \,\mu L$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area 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 sample 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 sample solution is not larger than 1/10 times the peak area of sarpogrelate from the standard solution, and the total area of the peaks other than sarpogrelate from the sample 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 after multiplying by the relative response factor, 0.78.

Operating conditions-

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

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

System suitability-

Test for required detectability: Pipet 5 mL of the standard solution, add the mobile phase to make exactly 50 mL. Confirm that the peak area of sarpogrelate with $10 \,\mu$ L of this solution is equivalent to 7 to 13% of that with $10 \,\mu$ L of the standard solution.

System performance: Dissolve 50 mg of Sarpogrelate Hydrochloride in 20 mL of water, and use as the sarpogrelate hydrochloride stock solution. To 1 mL of the sarpogrelate hydrochloride stock 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 $10 \,\mu$ L 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 $10 \,\mu\text{L}$ 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%.

(4) Residual solvent—Being specified separately.

Water <2.48> Not more than 0.5% (1 g, coulometric titration).

Residue on ignition $\langle 2.44 \rangle$ 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 $\langle 2.48 \rangle$ in the same manner as Sarpogrelate Hydrochloride), add to them exactly 2.5 mL of the internal standard solution, and dissolve them with the mobile phase to make 50 mL. To 5 mL each of these solutions add the mobile phase to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ 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.

Amount (mg) of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6$.HCl) = $M_S \times Q_T/Q_S$

 $M_{\rm S}$: Amount (mg) of Sarpogrelate Hydrochloride RS, calculated on the anhydrous basis

Internal standard solution—A solution of isopropyl parahydroxybenzoate in the mobile phase (3 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 272 nm).

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

Column temperature: A constant temperature of about $40^{\circ}C$.

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 μ L of the standard solution under the above operating conditions, sarpogrelate 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 $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of sarpogrelate to that of the internal standard is not more than 1.0%.

Containers and storage Containers-Tight containers.

Sarpogrelate Hydrochloride Fine Granules

サルポグレラート塩酸塩細粒

Sarpogrelate Hydrochloride Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6$.HCl: 465.97).

Method of preparation Prepare as directed under Granules, with Sarpogrelate Hydrochloride.

Identification To an amount of Sarpogrelate Hydrochloride Fine Granules, equivalent to 50 mg of Sarpogrelate Hydrochloride according to the labeled amount, add 10 mL of 0.01 mol/L hydrochloric acid TS, allow to stand at room temperature for 10 minutes, then add 0.01 mol/L hydrochloric acid TS to make 100 mL, and disperse the particles with the aid of ultrasonic waves. Centrifuge this solution, and to 5 mL of the supernatant liquid add 0.01 mol/L hydrochloric acid TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: it exhibits maxima between 269 nm and 273 nm and between 274 nm and 278 nm.

Purity Related substances—Perform the procedure within 3 hours after preparing the sample solution. Powder Sarpogrelate Hydrochloride Fine Granules. To a portion of the powder, equivalent to 0.10 g of Sarpogrelate Hydrochloride according to the labeled amount, add 50 mL of the mobile phase, and disperse the particles with the aid of ultrasonic waves. Filter thorough a membrane filter with a pore size not exceeding 0.45 μ m, discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly $10 \,\mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area 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 sample solution is not larger than 2.5 times that of sarpogrelate from the standard solution, and the area of the peak other than sarpogrelate and the peak mentioned above from the sample solution is not larger than 1/10 times the peak area of sarpogrelate from the standard solution. For this calculation use the peak area of the decomposed substance A after multiplying by the relative response factor, 0.78.

Operating conditions—

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

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

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 obtained with 10

 μ L of this solution is equivalent to 7 to 13% of that with 10 μ L of the standard solution.

System performance: Dissolve 50 mg of sarpogrelate hydrochloride in 20 mL of water, and use this solution as the sarpogrelate hydrochloride stock solution. To 1 mL of the sarpogrelate hydrochloride stock 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 μ L 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 $10 \,\mu\text{L}$ 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%.

Uniformity of dosage units <6.02> Perform the test according to the following method: Sarpogrelate Hydrochloride Fine Granules in single-unit containers meet the requirement of the Content uniformity test.

To the total amount of the content of 1 container of Sarpogrelate Hydrochloride Fine Granules add exactly V/10 mL of the internal standard solution, and add 4V/5 mL of the mobile phase, disperse the particles with the aid of ultrasonic waves, then add the mobile phase to make V mL so that each mL contains about 1 mg of sarpogrelate hydrochloride (C₂₄H₃₁NO₆.HCl), and centrifuge. To 5 mL of the supernatant liquid add the mobile phase to make 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6.HCl$) = $M_S \times Q_T/Q_S \times V/50$

 $M_{\rm S}$: Amount (mg) of Sarpogrelate Hydrochloride RS, calculated on the anhydrous basis

Internal standard solution—A solution of isopropyl parahydroxybenzoate in the mobile phase (1 in 1000).

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Sarpogrelate Hydrochloride Fine Granules is not less than 85%.

Start the test with an accuretely weighed amount of Sarpogrelate Hydrochloride Fine Granules, equivalent to about 50 mg of sarpogrelate hydrochloride (C₂₄H₃₁NO₆.HCl) according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μ m. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Sarpogrelate Hydrochloride RS (separately determine the water $\langle 2.48 \rangle$ in the same manner as Sarpogrelate Hydrochloride), and dissolve in water to make exactly 50 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, $A_{\rm T}$ and $A_{\rm S}$, of the sample solution and standard solution at 270 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6.HCl$)

 $= M_{\rm S}/M_{\rm T} \times A_{\rm T}/A_{\rm S} \times 1/C \times 180$

- $M_{\rm S}$: Amount (mg) of Sarpogrelate Hydrochloride RS, calculated on the anhydrous basis
- $M_{\rm T}$: Amount (g) of sample
- C: Labeled amount (mg) of sarpogrelate hydrochloride (C₂₄H₃₁NO₆.HCl) in 1 g

Particle size <6.03> It meets the requirements of Fine granules.

Assay Powder Sarpogrelate Hydrochloride Fine Granules. Weigh accurately a portion of the powder, equivalent to about 0.25 g of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6$. HCl), add exactly 25 mL of the internal standard solution, add about 200 mL of the mobile phase, and disperse the particles with the aid of ultrasonic waves. To this solution add the mobile phase to make 250 mL, and centrifuge. To 5 mL of the supernatant liquid add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Sarpogrelate Hydrochloride RS (separately determine the water $\langle 2.48 \rangle$ in the same manner as Sarpogrelate Hydroxide), add exactly 5 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 50 mL, and use this solution as the standard solution. Perform the test with $10 \,\mu L$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of sarpogrelate to that of the internal standard.

> Amount (mg) of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6.HCl$) = $M_S \times Q_T/Q_S \times 5$

 $M_{\rm S}$: Amount (mg) of Sarpogrelate Hydrochloride RS, calculated on the anhydrous basis

Internal standard solution—A solution of isopropyl parahydroxybenzoate in the mobile phase (1 in 1000). *Operating conditions*—

Proceed as directed in the operating conditions in the Assay under Sarpogrelate Hydrochloride.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, sarpogrelate 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 $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of sarpogrelate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Sarpogrelate Hydrochloride Tablets

サルポグレラート塩酸塩錠

Sarpogrelate Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6$. HCl: 465.97).

Method of preparation Prepare as directed under Tablets, with Sarpogrelate Hydrochloride.

Identification Powder Sarpogrelate Hydrochloride Tablets. To a portion of the powder, equivalent to 50 mg of Sarpogrelate Hydrochloride according to the labeled amount, add 10 mL of 0.01 mol/L hydrochloric acid TS, allow to stand at room temperature for 10 minutes, then add 0.01 mol/L hydrochloric acid TS to make 100 mL, and disperse the particles with the aid of ultrasonic waves. Centrifuge this solution, and to 5 mL of the supernatant liquid add 0.01 mol/L hydrochloric acid TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: it exhibits maxima between 269 nm and 273 nm, and between 274 nm and 278 nm.

Purity Related substances-Perform the procedure within 12 hours after preparing the sample solution. Powder Sarpogrelate Hydrochloride Tablets. To a portion of the powder, equivalent to 0.10 g of Sarpogrelate Hydrochloride according to the labeled amount, add 50 mL of the mobile phase, and disperse the particles with the aid of ultrasonic waves. Filter the solution through a membrane filter with a pore size not exceeding 0.45 μ m, discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly $10 \,\mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area 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 sample solution is not larger than 1.5 times that of sarpogrelate from the standard solution, and the area of the peak other than sarpogrelate and the peak mentioned above from the sample solution is not larger than 1/10 times the peak area of sarpogrelate from the standard solution. For this calculation use the peak area of the decomposed substance A after multiplying by the relative response factor, 0.78.

Operating conditions—

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

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

System suitability-

Test for required detectability: To exactly 5 mL of the standard solution add the mobile phase to make exactly 50 mL. Confirm that the peak area of sarpogrelate obtained with $10 \,\mu$ L of this solution is equivalent to 7 to 13% of that

with $10 \,\mu L$ of the standard solution.

System performance: Dissolve 50 mg of sarpogrelate hydrochloride in 20 mL of water, and use this solution as the sarpogrelate hydrochloride stock solution. To 1 mL of the stock 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 μ L 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 $10 \,\mu\text{L}$ 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%.

Uniformity of dosage units $\langle 6.02 \rangle$ Perform the test according to the following method: it meets the requirements of the Content uniformity test.

To 1 tablet of Sarpogrelate Hydrochloride Tablets add exactly V/10 mL of the internal standard solution, and disintegrate the tablet. Add 4V/5 mL of the mobile phase, disperse the particles with the aid of ultrasonic waves, then add the mobile phase to make V mL so that each mL contains about 1 mg of sarpogrelate hydrochloride (C₂₄H₃₁NO₆.HCl), and centrifuge. To 5 mL of the supernatant liquid add the mobile phase to make 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of sarpogrelate hydrochloride (C₂₄H₃₁NO₆.HCl) = $M_{\rm S} \times Q_{\rm T}/Q_{\rm S} \times V/50$

 $M_{\rm S}$: Amount (mg) of Sarpogrelate Hydrochloride RS, calculated on the anhydrous basis

Internal standard solution—A solution of isopropyl parahydroxybenzoate in the mobile phase (1 in 1000).

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Sarpogrelate Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Sarpogrelate Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μ m. 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 55.6 μ g of sarpogrelate hydrochloride $(C_{24}H_{31}NO_6.HCl)$ according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Sarpogrelate Hydrochloride RS (separately determine the water $\langle 2.48 \rangle$ in the same manner as Sarpogrelate Hydrochloride), and dissolve in water to make exactly 50 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, $A_{\rm T}$ and $A_{\rm S}$, of the sample solution and standard solution at 270 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6.HCl$) = $M_S \times A_T/A_S \times V'/V \times 1/C \times 180$

- $M_{\rm S}$: Amount (mg) of Sarpogrelate Hydrochloride RS, calculated on the anhydrous basis
- C: Labeled amount (mg) of sarpogrelate hydrochloride $(C_{24}H_{31}NO_6.HCl)$ in 1 tablet

Assay Weigh accurately the mass of not less than 20 Sarpogrelate Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.25 g of sarpogrelate hydrochloride (C24H31NO6.HCl), add exactly 25 mL of the internal standard solution, add about 200 mL of the mobile phase, and disperse the particles with the aid of ultrasonic waves. To this solution add the mobile phase to make 250 mL, and centrifuge. To 5 mL of the supernatant liquid add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Sarpogrelate Hydrochloride RS (separately determine the water $\langle 2.48 \rangle$ in the same manner as Sarpogrelate Hydroxide), add exactly 5 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 50 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> under the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of sarpogrelate to that of the internal standard.

> Amount (mg) of sarpogrelate hydrochloride ($C_{24}H_{31}NO_6.HCl$) = $M_S \times Q_T/Q_S \times 5$

 $M_{\rm S}$: Amount (mg) of Sarpogrelate Hydrochloride RS, calculated on the anhydrous basis

Internal standard solution—A solution of isopropyl parahydroxybenzoate in the mobile phase (1 in 1000). *Operating conditions*—

Proceed as directed in the operating conditions in the

Assay under Sarpogrelate Hydrochloride.

System suitability-

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, sarpogrelate 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 $10 \,\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio 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

ブチルスコポラミン臭化物



 $C_{21}H_{30}BrNO_4$: 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-9azoniatricyclo[3.3.1.0^{2,4}]nonane bromide [*149-64-4*]

Scopolamine Butylbromide, when dried, contains not less than 98.5% of $C_{21}H_{30}BrNO_4$.

Description Scopolamine Butylbromide occurs as white crystals or crystalline powder.

It 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 diethyl 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 spectrum of a solution of Scopolamine Butylbromide (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Scopolamine Butylbromide, previously dried, as directed in the potassium bromide disk method under Infrared Spectro-photometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Scopolamine Butylbromide (1 in 20) responds to the Qualitative Tests $\langle 1.09 \rangle$ for bromide.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_{D}^{20}$: $-18.0 - -20.0^{\circ}$ (after drying, 1 g, water, 10 mL, 100 mm).

pH $\langle 2.54 \rangle$ Dissolve 1.0 g of Scopolamine Butylbromide in 10 mL of water: the pH of this solution is between 5.5 and 6.5.

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 Matching Fluid F add diluted hydrochloric acid (1 in 40) to make 20 mL.

(2) Heavy metals <1.07>—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).

JP XVI

(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 sample solution. Separately, dissolve 10 mg of scopolamine hydrobromide in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution (1). Pipet 5 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 exactly 20 μ L each of the sample solution and standard solutions (1) and (2) as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the peak area of scopolamine from the sample 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 elution and the peak of scopolamine and butylscopolamine from the sample 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 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (10 μ m 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 the pH to 3.6 with diluted phosphoric acid (1 in 10).

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

Time span of measurement: About twice as long as the retention time of butylscopolamine.

System suitability—

System performance: Dissolve 5 mg each of Scopolamine Butylbromide and scopolamine hydrobromide in 50 mL of the mobile phase. When the procedure is run with $20 \,\mu$ L of this solution under the above operating conditions, scopolamine and butylscopolamine 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 $20 \,\mu\text{L}$ of the standard solution (2) under the above operating conditions, the relative standard deviation of the peak area of scopolamine is not more than 2.0%.

Loss on drying $\langle 2.41 \rangle$ Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.8 g of Scopolamine Butylbromide, previously dried, dissolve in 40 mL of acetic acid (100) and 30 mL of acetic anhydride, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 44.04 mg of C₂₁H₃₀BrNO₄

Containers and storage Containers—Tight containers.

Scopolamine Hydrobromide Hydrate

スコポラミン臭化水素酸塩水和物



C₁₇H₂₁NO₄.HBr.3H₂O: 438.31 (1*S*,2*S*,4*R*,5*R*,7*S*)-9-Methyl-3-oxa-9-azatricyclo-[3.3.1.0^{2,4}]non-7-yl (2*S*)-3-hydroxy-2-phenylpropanoate monohydrobromide trihydrate [6533-68-2]

Scopolamine Hydrobromide Hydrate, when dried, contains not less than 98.5% of scopolamine hydrobromide (C₁₇H₂₁NO₄.HBr: 384.26).

Description Scopolamine Hydrobromide Hydrate occurs as colorless or white crystals, or white granules or powder. It is odorless.

It is freely soluble in water, sparingly soluble in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl 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 $\langle 1.09 \rangle$ for bromide.

Optical rotation $\langle 2.49 \rangle$ [α]_D²⁰: -24.0 - -26.0° (after drying, 0.5 g, water, 10 mL, 100 mm).

Melting point $\langle 2.60 \rangle$ 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) Acidity—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 redmethylene 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 sample solution.

(i) To 1 mL of the sample solution add 2 to 3 drops of ammonia TS: no turbidity is produced.

(ii) To 1 mL of the sample 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 $\langle 2.41 \rangle$ 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 $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Scopolamine Hydrobromide Hydrate, previously dried, in 10 mL of acetic acid (100) by warming. After cooling, add 40 mL of acetic anhydride, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 38.43 mg of C₁₇H₂₁NO₄.HBr

Containers and storage Containers—Tight containers. Storage—Light-resistant.

L-Serine

L-セリン

C₃H₇NO₃: 105.09 (2*S*)-2-Amino-3-hydroxypropanoic acid [*56-45-1*]

L-Serine, when dried, contains not less than 98.5% and not more than 101.0% of $C_3H_7NO_3$.

Description L-Serine occurs as white crystals or a crystalline powder. It has a slight sweet taste.

It is freely soluble in water and in formic acid, and practically insoluble in ethanol (99.5).

It dissolves in 2 mol/L hydrochloric acid TS.

Identification Determine the infrared absorption spectrum of L-Serine as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_D^{20}$: +14.0 - +16.0° (After drying, 2.5 g, 2 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH $\langle 2.54 \rangle$ The pH of a solution prepared by dissolving 1.0 g of L-Serine in 10 mL of water is between 5.2 and 6.2.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of L-Serine in 10 mL of water: the solution is clear and color-less.

(2) Chloride $\langle 1.03 \rangle$ —Perform the test with 0.5 g of L-Serine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate $\langle 1.14 \rangle$ —Perform the test with 0.6 g of L-Serine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium $\langle 1.02 \rangle$ —Perform the test with 0.25 g of L-Serine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <1.07>—Proceed with 2.0 g of L-Serine 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 $\langle 1.10 \rangle$ —Prepare the test solution with 1.0 g of L-Serine according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Dissolve 0.10 g of L-Serine in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 10 mL. 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 under Thinlayer Chromatography $\langle 2.03 \rangle$. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Then develop with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in a mixture of methanol and acetic acid (100) (97:3) (1 in 100) on the plate, and heat at 80°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.3% (1 g, 105°C, 3 hours).

Residue on Ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.11 g of L-Serine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = $10.51 \text{ mg of } C_3H_7NO_3$

Containers and storage Containers—Tight containers.

Serrapeptase

セラペプターゼ

[95077-02-4]

Serrapeptase is the enzyme preparation having proteolytic activity, produced by the growth of *Serratia* species.

Usually, it is diluted with Lactose Hydrate.

It contains not less than 2000 serrapeptase Units and not more than 2600 serrapeptase Units per mg.

It is hygroscopic.

Description Serrapeptase occurs as a grayish white to light brown powder, having a slight characteristic odor.

Identification Dissolve 0.4 g of Serrapeptase in 100 mL of acetic acid-sodium acetate buffer solution, pH 5.0, transfer exactly 1 mL each of this solution into three tubes, and refer to them as A, B and C. To tube A add exactly 1 mL of water, to tubes B and C add exactly 1 mL of 0.04 mol/L disodium dihydrogen ethylenediamine tetraacetate TS, mix gently, and allow them to stand in a water bath at $4 \pm 1^{\circ}$ C for about 1 hour. Then, to the tube B add exactly 2 mL of 0.04 mol/L zinc chloride TS, to the tubes A and C add ex-

actly 2 mL of water, mix gently, and allow them to stand in a water bath at 4 ± 1 °C for about 1 hour. Pipet 1 mL each of these solutions, add borate-hydrochloric acid buffer solution, pH 9.0 to the solutions A and B to make exactly 200 mL, to the solution C to make exactly 50 mL, and use these solutions as the sample solutions. Proceed with these sample solutions as directed in the Assay: the activities of the solutions A and B are almost the same, and the activity of the solution C is not more than 5% of that of the solution A.

Activity of solutions A, B or C = $A_T/A_S \times 1/20 \times D \times 176$

 $A_{\rm S}$: Absorbance of the standard solution

 $A_{\rm T}$: Absorbance of the sample solution

20: Reaction time (minute)

- D: Dilution rate (200 for solution A and B, 50 for solution C)
- 176: Conversion factor (Total volume of enzyme reaction solution/volume of filtrate taken × amount of tyrosine in 2 mL of tyrosine standard solution)

Purity (1) Heavy metals $\langle 1.07 \rangle$ —Put 1.0 g of Serrapeptase in a porcelain crucible, add 2 drops each of sulfuric acid and nitric acid, and incinerate by ignition. After cooling, to the residue add 2 mL of hydrochloric acid, evaporate to dryness on a water bath, add 10 mL of a solution of hydroxylamine hydrochloride (3 in 100) and 2 mL of dilute acetic acid, and heat on a water bath for 5 minutes. After cooling, filter if necessary, wash the filter paper with 10 mL of water, put the filtrate and washing in a Nessler tube, add water to make 50 mL, and use this solution as the test solution. Prepare the control solution as follows: Evaporate to dryness 2 drops each of sulfuric acid and nitric acid on a sand bath, add 2 mL of hydrochloric acid to the residue, evaporate to dryness on a water bath, add 2.0 mL of Standard Lead Solution, 10 mL of a solution of hydroxylamine hydrochloride (3 in 100) and 2 mL of dilute acetic acid, and heat on a water bath for 5 minutes. Proceed in the same manner as directed for the preparation of the test solution, and add water to make 50 mL (not more than 20 ppm).

(2) Arsenic $\langle 1.11 \rangle$ —Prepare the test solution with 0.40 g of Serrapeptase according to Method 3, excepting addition of 5 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (3 in 10) instead of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), evaporating to dryness on a water bath, then incinerating with a small flame, and perform the test (not more than 5 ppm).

Loss on drying $\langle 2.41 \rangle$ Not more than 7.0% (1 g, 105°C, 4 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 1.5% (1 g).

Assay (i) Sample solution: Dissolve exactly 0.100 g of Serrapeptase in a solution of ammonium sulfate (1 in 20) to make exactly 100 mL. Pipet 1 mL of this solution, add borate-hydrochloric acid buffer solution, pH 9.0 to make exactly 200 mL, and use this solution as the sample solution.

(ii) Tyrosine standard solution: Dissolve exactly 0.160 g of Tyrosine RS, previously dried at $105 \,^{\circ}$ C for 3 hours, in 0.2 mol/L hydrochloric acid TS to make exactly 1000 mL. Pipet 10 mL of this solution, and add 0.2 mol/L hydrochloric acid TS to make exactly 100 mL. Prepare before use.

(iii) Substrate solution: Previously determine the loss on drying $\langle 2.41 \rangle$ (60°C, reduced pressure not exceeding 0.67 kPa, 3 hours) of milk casein, previously dried. To exactly

1.20 g of the milk casein, calculated based on the loss on drying, add 160 mL of a solution of sodium borate (19 in 1000), and heat in a water bath to dissolve. After cooling, adjust the pH to exactly 9.0 with 1 mol/L hydrochloric acid TS, and add borate-hydrochloric acid buffer solution, pH 9.0 to make exactly 200 mL. Use after warming to $37 \pm 0.5^{\circ}$ C. Prepare before use.

(iv) Precipitation reagent: Trichloroacetic acid TS for serrapeptase. Use after warming to $37 \pm 0.5^{\circ}$ C.

(v) Procedure: Pipet 1 mL of the sample solution, put in a glass-stoppered tube (15×130 mm), allow to stand at 37 ± 0.5 °C for 5 minutes, add exactly 5 mL of the substrate solution, and mix well immediately. Allow to stand at 37 \pm 0.5°C for exactly 20 minutes, add exactly 5 mL of trichloroacetic acid TS for serrapeptase, mix, allow to stand at $37 \pm 0.5^{\circ}$ C for 30 minutes, and filter through a dried filter paper. Pipet 2 mL of the filtrate, add exactly 5 mL of a solution of anhydrous sodium carbonate (3 in 50), mix, add exactly 1 mL of diluted Folin's TS (1 in 3), mix well, and allow to stand at 37 ± 0.5 °C for 30 minutes. Determine the absorbance of this solution at 660 nm, A_1 , as directed under Ultraviolet-visible Spectrophotometry <2.24> using water as the blank. Separately, pipet 1 mL of the sample solution, add exactly 5 mL of trichloroacetic acid TS for serrapeptase, mix, add exactly 5 mL of the substrate solution, allow to stand at 37 ± 0.5 °C for 30 minutes, and proceed in the same manner as directed above to determine the absorbance A_2 . Separately, pipet 2 mL of the tyrosine standard solution, add exactly 5 mL of a solution of anhydrous sodium carbonate (3 in 50), mix, add exactly 1 mL of diluted Folin's TS (1 in 3), mix well, and proceed in the same manner as directed above to determine the absorbance A_3 . Separately, pipet 2 mL of 0.2 mol/L hydrochloric acid TS, and proceed in the same manner as directed above to determine the absorbance A_4 .

Serrapeptase Unit per mg of Serrapeptase
=
$$(A_1 - A_2)/(A_3 - A_4) \times 1/20 \times 200 \times 176$$

20: Reaction time (minute)

200: Dilution rate

176: Conversion factor (Total volume of enzyme reaction solution/volume of filtrate taken × amount of tyrosine in 2 mL of tyrosine standard solution)

One serrapeptase Unit corresponds to the amount of serrapeptase which produces $1 \mu g$ of tyrosine per minute from 5 mL of the substrate solution under the above conditions.

Containers and storage Containers—Tight containers.

Sesame Oil

Oleum Sesami

ゴマ油

Sesame Oil is the fixed oil obtained from the seeds of *Sesamum indicum* Linné (*Pedaliaceae*).

Description Sesame Oil is a clear, pale yellow oil. It is odorless or has a faint, characteristic odor, and has a bland taste.

It is miscible with diethyl ether and with petroleum ether. It is slightly soluble in ethanol (95). It congeals between 0°C and -5°C. Congealing point of the fatty acids: 20 - 25°C

Identification To 1 mL of Sesame Oil add 0.1 g of sucrose and 10 mL of hydrochloric acid, and shake for 30 seconds: the acid layer becomes light red and changes to red on standing.

Specific gravity <1.13> d_{20}^{20} : 0.914 - 0.921

Acid value <1.13> Not more than 0.2.

Saponification value <1.13> 187 – 194

Unsaponifiable matters <1.13> Not more than 2.0%.

Iodine value <1.13> 103 – 118

Containers and storage Containers—Tight containers.

Sevoflurane

セボフルラン



C₄H₃F₇O: 200.05

1,1,1,3,3,3-Hexafluoro-2-(fluoromethoxy)propane [28523-86-6]

Sevoflurane contains not less than 99.0% and not more than 101.0% of $C_4H_3F_7O$, calculated on the anhydrous basis.

Description Sevoflurane is a clear, colorless, and mobile liquid.

It is miscible with ethanol (99.5).

It is very slightly soluble in water.

It is volatile and inflammable.

Refractive index n_D^{20} : 1.2745 – 1.2760 Boiling point: about 58.6°C.

Bolling point. about 58.0 C.

Identification Transfer about $1 \mu L$ of Sevoflurane to a gas cell having light path 10 cm in length, and determine the infrared absorption spectrum as directed in the gas sampling method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Sevoflurane RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific gravity <2.56> d_{20}^{20} : 1.510 – 1.530

Purity (1) Acidity or alkalinity—To 50 mL of Sevoflurane with 50 mL of freshly boiled and cooled water vigorously for 3 minutes. Separate the water layer and use this solution as the sample solution. To 20 mL of the sample solution add 1 drop of bromocresol purple TS and 0.10 mL of 0.01 mol/L sodium hydroxide VS: a red-purple color develops. To 20 mL of the sample solution add 1 drop of bromocresol purple TS and 0.6 mL of 0.01 mol/L hydrochloric acid VS: a yellow color is produced.

(2) Soluble fluoride—To 6 g of Sevoflurane add 12 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20), and shake for 10 minutes. Transfer 4.0 mL of diluted 0.01 mol/L sodium hydroxide solution (1 in 20) layer into a Nessler tube.

Add 30 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3 and cerium (III) nitrate TS (1:1:1), add water to make 50 mL, allow to stand for 60 minutes, and use this solution as the sample solution. Separately, transfer 0.2 mL of the fluorine standard solution and 4.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) into a Nessler tube, and add 30 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3 and cerium (III) nitrate TS (1:1:1). Proceed in the same manner as directed for the preparation of the sample solution, and use this solution as the standard solution. Determine the absorbances of the sample solution and standard solution at 600 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared with 4.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) in the same manner, as the blank: the absorbance of the sample solution is not more than that of the standard solution (not more than 1 ppm).

Fluorine standard solution: Dissolve exactly 2.21 g of sodium fluoride in water to make exactly 1000 mL. Pipet 10 mL of this solution and add water to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of fluorine (F).

(3) Related substances—Perform the test with $2 \mu L$ of Sevoflurane as directed under Gas Chromatography $\langle 2.02 \rangle$ 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 the peak of hexafluoroisopropyl methyl ether, having the relative retention time of about 0.84 with respect to sevoflurane, is not more than 0.005%, the amount of each peak other than the peaks of sevoflurane and hexafluoroisopropyl methyl ether is not more than 0.0025%, and the total amount of the peaks other than the peaks of sevoflurane and hexafluoroisopropyl methyl ether is not more than 0.005%. *Operating conditions*—

Detector, column, injection port temperature, detector temperature, carrier gas and split ratio: Proceed as directed in the operating conditions in the Assay.

Column temperature: Inject at a constant temperature of about 40°C, maintain the temperature for 10 minutes, raise at a rate of 10°C per minute to 200°C, and maintain at a constant temperature of about 200°C.

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

Time span of measurement: About 6 times as long as the retention time of sevoflurane.

System suitability—

Test for required detectability: To $20 \,\mu\text{L}$ of Sevoflurane add *o*-xylene to make 20 mL. To 1 mL of this solution add *o*-xylene to make 20 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test and add *o*-xylene to make exactly 10 mL. Confirm that the peak area of sevoflurane obtained from 2 μL of this solution is equivalent to 7 to 13% of the peak area of sevoflurane from 2 μ L of the solution for system suitability test.

System performance: When the procedure is run with $2 \mu L$ of the solution for system suitability test under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of sevoflurane are not less than 6000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with $2 \mu L$ of the solution for system suitability test under the

JP XVI

above operating conditions, the relative standard deviation of the peak area of Sevoflurane is not more than 5.0%.

(4) Residual solvent—Being specified separately.

(5) Residue on evaporation—Evaporate 10 mL of Sevoflurane, exactly measured, on a water bath to dryness, and dry at 105° C for 2 hours: the mass of the residue is not more than 1.0 mg.

Water $\langle 2.48 \rangle$ Not more than 0.2 w/v% (5 mL, volumetric titration, direct titration).

Assay Pipet 5 mL each of Sevoflurane and Sevoflurane RS (separately determine the water <2.48> using the same manner as Sevoflurane), to each add exactly 5 mL of dimethoxymethane as an internal standard, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 1 μ L each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of sevoflurane to that of the internal standard.

Amount (mg) of sevoflurane (C₄H₃F₇O) = $V_{\rm S} \times Q_{\rm T}/Q_{\rm S} \times 1000 \times 1.521$

 $V_{\rm S}$: Amount (mL) of Sevoflurane RS, calculated on the anhydrous basis

1.521: Specific gravity of Sevoflurane (d_{20}^{20})

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.32 mm in inside diameter and 30 m in length, coated inside with cyanopropyl methylphenyl silicone for gas chromatography in $1.8 \,\mu$ m thickness.

Column temperature: 40°C.

Injection port temperature: A constant temperature of about 200°C.

Detector temperature: A constant temperature of about 225°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of Sevoflurane is about 3 minutes.

Split ratio: 1:20.

System suitability-

System performance: When the procedure is run with $1 \mu L$ of the standard solution under the above operating conditions, sevoflurane 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 $1 \mu L$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of sevoflurane to that of the internal standard is not more than 1.0 %.

Containers and storage Containers—Tight containers.

Purified Shellac

精製セラック

Purified Shellac is a resin-like substance obtained from a purified secretion of *Laccifer lacca* Kerr (*Coccidae*).

Description Purified Shellac occurs as light yellow-brown to brown, lustrous, hard, brittle scutella. It has no odor or has a faint, characteristic odor.

It is freely soluble in ethanol (95) and in ethanol (99.5), and practically insoluble in water and in diethyl ether.

It dissolves in sodium hydroxide TS.

Acid value $\langle 1.13 \rangle$ 60 – 80 Weigh accurately about 1 g of Purified Shellac, add 40 mL of neutralized ethanol, and dissolve by warming. After cooling, titrate $\langle 2.50 \rangle$ with 0.1 mol/L potassium hydroxide VS (potentiometric titration).

Purity (1) Heavy metals $\langle 1.07 \rangle$ —Proceed with 2.0 g of Purified Shellac 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 $\langle 1.11 \rangle$ —Prepare the test solution with 0.40 g of Purified Shellac according to Method 3, and perform the test. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and fire to burn (not more than 5 ppm).

(3) Ethanol-insoluble substances—Dissolve about 5 g of Purified Shellac, accurately weighed, in 50 mL of ethanol (95) on a water bath while shaking. Pour the ethanol solution into a tared extraction thimble, previously dried at 105° C for 2 hours, in a Soxhlet extractor, and extract with ethanol (95) for 3 hours. Dry the extraction thimble at 105° C for 3 hours: the mass of the residue is not more than 2.0%. Use a cylindrical weighing bottle for taring the extraction thimble.

(4) Rosin—Dissolve 2.0 g of Purified Shellac in 10 mL of ethanol (99.5) with thorough shaking, add gradually 50 mL of petroleum ether while shaking, and filter, if necessary. Wash the solution with two 50-mL portions of water, filter the upper layer, and evaporate the filtrate on a water bath to dryness. Dissolve the residue in 2 mL of a mixture of carbon tetrachloride and phenol (2:1), transfer the solution to a depression of a spot plate, and fill the neighboring depression with a mixture of carbon tetrachloride and bromine (4:1). Immediately cover both depressions with a watch glass, and allow to stand: the solution of the residue exhibits no purple or blue color within 1 minute.

(5) Wax—Dissolve 10.0 g of Purified Shellac in 150 mL of a solution of sodium carbonate decahydrate (9 in 200) with shaking on a water bath, and continue the heating for 2 hours. After cooling, collect the floating wax by filtration, wash the wax and the filter paper with water, transfer to a beaker, and dry at 65° C until the water is almost evaporated. Transfer the wax together with the filter paper to an extraction thimble in a Soxhlet extractor. Dissolve the wax remaining in the beaker with a suitable quantity of chloroform by warming. Pour the solution into the thimble, and extract with chloroform for 2 hours. Evaporate the chloroform solution to dryness, ad dry the residue at 105°C for 3 hours: the mass of the residue is not more than 20 mg.

Loss on drying Not more than 2.0%. Weigh accurately about 1 g of medium powder of Purified Shellac, and dry at 40° C for 4 hours, then for 15 hours in a desiccator (calcium chloride for drying).

Total ash $\langle 5.01 \rangle$ Not more than 1.0% (1 g).

Containers and storage Containers—Well-closed containers.

White Shellac

白色セラック

White Shellac is a resin-like substance obtained from a bleached secretion of *Laccifer lacca* Kerr (*Coccidae*).

Description White Shellac occurs as yellowish white to light yellow, hard, brittle granules. It is odorless or has a faint, characteristic odor.

It is sparingly soluble in ethanol (95), very slightly soluble in petroleum ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Acid value $\langle 1.13 \rangle$ 65 – 90 Weigh accurately about 0.5 g of White Shellac, add 50 mL of neutralized ethanol as a solvent, and dissolve by warming. After cooling, perform the test as directed in the Acid value under Purified Shellac.

Purity (1) Chloride $\langle 1.03 \rangle$ —Shake and dissolve 0.40 g of White Shellac in 5 mL of ethanol (95) while warming, add 40 mL of water, and cool. Add 12 mL of dilute nitric acid and water to make 100 mL, and filter. Perform the test using 50 mL of the filtrate as the test solution. Prepare the control solution as follows: to 0.80 mL of 0.01 mol/L hydrochloric acid VS add 2.5 mL of ethanol (95), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.140%).

(2) Sulfate $\langle 1.14 \rangle$ —Shake and dissolve 0.40 g of White Shellac in 5 mL of ethanol (95) by warming, add 40 mL of water, and cool. Add 2 mL of dilute hydrochloric acid and water to make 100 mL, and filter. Perform the test using 50 mL of the filtrate as the test solution. Prepare the control solution as follows: to 0.45 mL of 0.005 mol/L sulfuric acid VS add 2.5 mL of ethanol (95), 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.110%).

(3) Heavy metals $\langle 1.07 \rangle$ —Proceed with 2.0 g of White Shellac 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 $\langle 1.11 \rangle$ —Prepare the test solution with 0.40 g of White Shellac according to Method 3, and perform the test. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and fire to burn (not more than 5 ppm).

(5) Ethanol-insoluble substances—Dissolve about 5 g of White Shellac, accurately weighed, in 50 mL of ethanol (95) on a water bath while shaking. Pour the ethanol solution into a tared extraction thimble, previously dried at $105 \,^{\circ}$ C for 2 hours, in a Soxhlet extractor, and extract with ethanol (95) for 3 hours. Dry the extraction thimble at $105 \,^{\circ}$ C for 3 hours: the mass of the residue is not more than 2.0%. Use a cylindrical weighing bottle for taring the extraction thimble.

(6) Rosin—Dissolve 2.0 g of White Shellac in 10 mL of

ethanol (99.5) with thorough shaking, add gradually 50 mL of petroleum ether while shaking, and filter, if necessary. Wash the solution with two 50-mL portions of water, filter the upper layer, and evaporate the filtrate on a water bath to dryness. Dissolve the residue in 2 mL of a mixture of carbon tetrachloride and phenol (2:1), transfer the solution to a depression of a spot plate, and fill the neighboring depression with a mixture of carbon tetrachloride and bromine (4:1). Immediately cover both depressions with a watch glass, and allow to stand: the solution of the residue exhibits no purple or blue color within 1 minute.

(7) Wax—Dissolve 10.0 g of White Shellac in 150 mL of a solution of sodium carbonate decahydrate (9 in 200) with shaking on a water bath, and continue the heating for 2 hours. After cooling, collect the floating wax by filtration, wash the wax and the filter paper with water, transfer to a beaker, and dry at 65° C until the water is almost evaporated. Transfer the wax together with the filter paper to an extraction thimble in a Soxhlet extractor. Dissolve the wax remaining in the beaker with a suitable quantity of chloroform by warming. Pour the solution into the thimble, and extract with chloroform for 2 hours. Evaporate the chloroform solution to dryness, and dry the residue at 105° C for 3 hours: the mass of the residue is not more than 20 mg.

Loss on drying Not more than 6.0%. Weigh accurately about 1 g of medium powder of White Shellac, and dry at 40°C for 4 hours, then for 15 hours in a desiccator (calcium chloride for drying).

Total ash $\langle 5.01 \rangle$ Not more than 1.0% (1 g).

Containers and storage Containers—Well-closed containers.

Storage-In a cold place.

Siccanin

シッカニン



 $C_{22}H_{30}O_3$: 342.47 (4aS,6aS,11bR,13aS,13bS)-4,4,6a,9-Tetramethyl-1,2,3,4,4a,5,6,6a,11b,13b-decahydro-13*H*benzo[*a*]furo[2,3,4-*mn*]xanthen-11-ol [22733-60-4]

Siccanin is a substance having antifungal activity produced by the growth of *Helminthosporium siccans*.

It contains not less than $980 \,\mu g$ (potency) and not more than $1010 \,\mu g$ (potency) per mg, calculated on the dried basis. The potency of Siccanin is expressed as mass (potency) of siccanin ($C_{22}H_{30}O_3$).

Description Siccanin occurs as white to light yellow, crystals or crystalline powder.

It is freely soluble in acetone, soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a

solution of Siccanin in ethanol (99.5) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Siccanin RS obtained in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Siccanin as directed in the potassium bromide disk method under the Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Siccanin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_D^{20}$: $-165 - -175^{\circ}$ (0.1 g, ethanol (99.5), 10 mL, 100 mm).

Melting point <2.60> 138 – 142°C

Purity (1) Heavy metals $\langle 1.07 \rangle$ —Proceed with 1.0 g of Siccanin 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 0.20 g of Siccanin in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thinlayer Chromatography $\langle 2.03 \rangle$. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and acetone (5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-chlorobenzenedia-zonium TS on the plate: the number of the spots other than the principal spot obtained from the sample solution is not more than three, and they are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, reduced pressure not exceeding 0.67 kPa, 80°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately an amount of Siccanin and Siccanin RS, equivalent to about 50 mg (potency), dissolve each in the internal standard solution to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of siccanin to that of the internal standard.

Amount [
$$\mu$$
g (potency)] of C₂₂H₃₀O₃
= $M_{\rm S} \times Q_{\rm T}/Q_{\rm S} \times 1000$

M_S: Amount [mg (potency)] of Siccanin RS

Internal standard solution—A solution of 1,4-diphenylbenzene in methanol (1 in 30,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-length: 280 nm).

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

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

Mobile phase: A mixture of methanol and phosphate buffer solution, pH 5.9 (19:6).

Flow rate: Adjust the flow rate so that the retention time of siccanin is about 17 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, siccanin 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 $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of siccanin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Light Anhydrous Silicic Acid

軽質無水ケイ酸

Light Anhydrous Silicic Acid, calculated on the incinerated basis, contains not less than 98.0% of silicon dioxide (SiO₂: 60.08).

Description Light Anhydrous Silicic Acid occurs as a white to bluish white, light, fine power. It is odorless and tasteless, and smooth to the touch.

It is practically insoluble in water, in ethanol (95), and in diethyl ether.

It dissolves in hydrofluoric acid, in hot potassium hydroxide TS and in hot sodium hydroxide TS, and does not dissolve in dilute hydrochloric acid.

Identification (1) Dissolve 0.1 g of Light Anhydrous Silicic Acid in 20 mL of sodium hydroxide TS by boiling, and add 12 mL of ammonium chloride TS: a white, gelatinous precipitate is produced. The precipitate does not dissolve in dilute hydrochloric acid.

(2) To the precipitate obtained in (1) add 10 mL of a solution of methylene blue trihydrate (1 in 10,000), and wash with water: the precipitate has a blue color.

(3) Prepare a bead by fusing ammonium sodium hydrogenphosphate tetrahydrate on a platinum loop. Bring the hot, transparent bead into contact with Light Anhydrous Silicic Acid, and fuse again: an insoluble matter is perceptible in the bead. The resulting bead, upon cooling, becomes opaque and acquires a reticulated appearance.

Purity (1) Chloride $\langle 1.03 \rangle$ —Dissolve 0.5 g of Light Anhydrous Silicic Acid in 20 mL of sodium hydroxide TS by boiling, cool, filter if necessary, and wash with 10 mL of water. Combine the filtrate and washings, add 18 mL of dilute nitric acid, shake, and add water to make 50 mL. Perform the test using this solution as the test solution. To 0.15 mL of 0.01 mol/L hydrochloric acid VS add 20 mL of sodium hydroxide TS, 18 mL of dilute nitric acid and water to make 50 mL, and use this solution as the control solution (not more than 0.011%).

(2) Heavy metals <1.07>—Dissolve 0.5 g of Light Anhydrous Silicic Acid in 20 mL of sodium hydroxide TS by boiling, cool, add 15 mL of acetic acid (31), shake, filter if necessary, wash with 10 mL of water, combine the filtrate and washings, and add water to make 50 mL. Perform the test using this solution as the test solution. Add acetic acid (31) to 20 mL of sodium hydroxide TS and 1 drop of phenol-phthalein TS until the color of this solution disappears, add 2.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid and water to make 50 mL, and use this solution as the control solution (not more than 40 ppm).

(3) Iron $\langle 1.10 \rangle$ —To 40 mg of Light Anhydrous Silicic Acid add 10 mL of dilute hydrochloric acid, and heat for 10 minutes in a water bath while shaking. After cooling, add 0.5 g of L-tartaric acid to dissolve by shaking. Prepare the test solution with this solution according to Method 2, and perform the test according to Method B. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 500 ppm).

(4) Aluminum—Dissolve 0.5 g of Light Anhydrous Silicic Acid in 40 mL of sodium hydroxide TS by boiling, cool, add sodium hydroxide TS to make 50 mL, and filter. Measure 10 mL of the filtrate, add 17 mL of acetic acid (31), shake, add 2 mL of aluminon TS and water to make 50 mL, and allow to stand for 30 minutes: the color of this solution is not deeper than that of the following control solution.

Control solution: Dissolve 0.176 g of aluminum potassium sulfate dodecahydrate in water, and add water to make 1000 mL. To 15.5 mL of this solution add 10 mL of sodium hydroxide TS, 17 mL of acetic acid (31), 2 mL of aluminon TS and water to make 50 mL.

(5) Calcium—Dissolve 1.0 g of Light Anhydrous Silicic Acid in 30 mL of sodium hydroxide TS by boiling, cool, add 20 mL of water, 1 drop of phenolphthalein TS and dilute nitric acid until the color of this solution disappears, immediately add 5 mL of dilute acetic acid, shake, add water to make 100 mL, and obtain a clear liquid by centrifugation or filtration. To 25 mL of this liquid add 1 mL of oxalic acid TS and ethanol (95) to make 50 mL, immediately shake, and allow to stand for 10 minutes: the turbidity of this solution is not deeper than that of the following control solution.

Control solution: Dissolve 0.250 g of calcium carbonate, previously dried at 180 °C for 4 hours, in 3 mL of dilute hydrochloric acid, and add water to make 100 mL. To 4 mL of this solution add 5 mL of dilute acetic acid and water to make 100 mL. To 25 mL of this solution add 1 mL of oxalic acid TS and ethanol (95) to make 50 mL, and shake.

(6) Arsenic $\langle 1.11 \rangle$ —Dissolve 0.40 g of Light Anhydrous Silicic Acid in 10 mL of sodium hydroxide TS by boiling in a porcelain crucible, cool, add 5 mL of water and 5 mL of dilute hydrochloric acid, shake, and perform the test with this solution as the test solution (not more than 5 ppm).

Loss on drying $\langle 2.41 \rangle$ Not more than 7.0% (1 g, 105°C, 4 hours).

Loss on ignition $\langle 2.43 \rangle$ Not more than 12.0% (1 g, 850 - 900°C, constant mass).

Volume test Weigh 5.0 g of Light Anhydrous Silicic Acid, transfer gradually to a 200-mL measuring cylinder, and allow to stand: the volume is not less than 70 mL.

Assay Weigh accurately about 1 g of Light Anhydrous Silicic Acid, add 20 mL of hydrochloric acid, and evaporate to dryness on a sand bath. Moisten the residue with hydro-chloric acid, evaporate to dryness, and heat between 110°C and 120°C for 2 hours. Cool, add 5 mL of dilute hydrochlo-

ric acid, and heat. Allow to cool to room temperature, add 20 to 25 mL of hot water, filter rapidly, and wash the residue with warm water until the last washing becomes negative to the Qualitative Tests $\langle 1.09 \rangle$ (2) for chloride. Transfer the residue together with the filter paper to a platinum crucible, ignite to ash, and continue the ignition for 30 minutes. Cool, weigh the crucible, and designate the mass as *a* (g). Moisten the residue in the crucible with water, add 6 mL of hydro-fluoric acid and 3 drops of sulfuric acid, and evaporate to dryness. Heat strongly for 5 minutes, cool, weigh the crucible, and designate the mass as *b* (g).

Content (g) of silicon dioxide (SiO₂) = a - b

Containers and storage Containers—Tight containers.

Silver Nitrate

硝酸銀

AgNO₃: 169.87

Silver Nitrate, when dried, contains not less than 99.8% of AgNO₃.

Description Silver Nitrate occurs as lustrous, colorless or white crystals.

It is very soluble in water, soluble in ethanol (95), and practically insoluble in diethyl ether.

It gradually turns grayish black by light.

Identification A solution of Silver Nitrate (1 in 50) responds to the Qualitative Tests <1.09> 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 freshly boiled and cooled water: the solution is clear and colorless. It is 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 $\langle 2.41 \rangle$ Not more than 0.20% (2 g, silica gel, light resistant, 4 hours).

Assay Weigh accurately about 0.7 g of Silver Nitrate, previously powdered and dried, dissolve in 50 mL of water, add 2 mL of nitric acid, and titrate $\langle 2.50 \rangle$ 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 = $16.99 \text{ mg of AgNO}_3$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Silver Nitrate Ophthalmic Solution

硝酸銀点眼液

Silver Nitrate Ophthalmic Solution is an aqueous eye lotion.

It contains not less than 0.95 w/v% and not more than 1.05 w/v% of silver nitrate (AgNO₃: 169.87).

Method of preparation

Silver Nitrate	10 g
Purified Water or Purified	
Water in Containers	a sufficient quantity
	To make 1000 mL

Prepare as directed under Ophthalmic Preparations, with the above ingredients.

Description Silver Nitrate Ophthalmic Solution is a clear, colorless liquid.

Identification Silver Nitrate Ophthalmic Solution responds to the Qualitative Tests <1.09> for silver salt and for nitrate.

Assay Measure accurately 20 mL of Silver Nitrate Ophthalmic Solution, add 30 mL of water and 2 mL of nitric acid, and titrate $\langle 2.50 \rangle$ 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 = 16.99 mg of AgNO₃

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Silver Protein

プロテイン銀

Silver Protein is a compound of silver and proteins. It contains not less than 7.5% and not more than 8.5% of silver (Ag: 107.87).

Description Silver Protein occurs as a light yellow-brown to brown powder. It is odorless.

It (1 g) dissolves slowly in 2 mL of water. It is practically insoluble in ethanol (95), in diethyl ether and in chloroform.

The pH of a solution of Silver Protein (1 in 10) is between 7.0 and 8.5.

It is slightly hygroscopic.

It 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 develops.

(2) To 5 mL of a solution of Silver Protein (1 in 100) add dropwise 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 strong heating, dissolve the residue in 1 mL of nitric acid by warming, and

add 10 mL of water: this solution responds to the Qualitative Tests $\langle 1.09 \rangle$ (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 100-mL decomposition flask, add 10 mL of sulfuric acid, cover the flask with a funnel, and boil for 5 minutes. Cool, add dropwise 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. After cooling, transfer the solution to a 250-mL conical flask with 100 mL of water, and titrate $\langle 2.50 \rangle$ 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 = 10.79 mg of Ag

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Silver Protein Solution

プロテイン銀液

Silver Protein Solution contains not less than 0.22 w/v% and not more than 0.26 w/v% of silver (Ag: 107.87).

Method of preparation

Silver Protein	30 g	
Glycerin Mentha Water	a sufficient quantity	
	To make 1000 mL	

Dissolve and mix the above ingredients.

Description Silver Protein Solution is a clear, brown liquid, having the odor of mentha oil.

Identification (1) To 1 mL of Silver Protein Solution add 10 mL of ethanol (95), mix, and add 2 mL of sodium hydroxide TS. Add immediately 1 mL of a solution of copper (II) chloride dihydrate in ethanol (95) (1 in 10), shake, and filter: the filtrate is blue in color (glycerin).

(2) To 3 mL of Silver Protein Solution add water to make 10 mL, add 2 mL of dilute hydrochloric acid, shake frequently for 5 minutes, and filter. Add 5 mL of a solution of sodium hydroxide (1 in 10) to the filtrate, and add 2 mL of diluted copper (II) sulfate TS (2 in 25): a purple color develops (silver protein).

(3) To 5 mL of the sample solution obtained in (2) add iron (III) chloride TS dropwise: a brown precipitate is formed (silver protein).

(4) Place 3 mL of Silver Protein Solution in a crucible, heat cautiously, and evaporate almost to dryness. Then incinerate gradually by strong heating, dissolve the residue in 1 mL of nitric acid by warming, and add 10 mL of water: the solution responds to the Qualitative Tests $\langle 1.09 \rangle$ (1) for silver salt.

1388 Simple Ointment / Official Monographs

Assay Pipet 25 mL of Silver Protein Solution into a 250-mL Kjeldahl flask, and heat cautiously until a white gas of glycerin is evolved. After cooling, add 25 mL of sulfuric acid, cover the flask with a funnel, and heat gently for 5 minutes. After cooling, drop gradually 5 mL of nitric acid, heat with occasional shaking in a water bath for 45 minutes, and cool. Add 2 mL of nitric acid, boil gently, and repeat this operation until the solution becomes colorless upon cooling. Transfer cautiously the cooled content in the flask into a 500-mL conical flask with 250 mL of water. Boil gently for 5 minutes, cool, and titrate $\langle 2.50 \rangle$ 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 = 10.79 mg of Ag

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Simple Ointment

単軟膏

Method of preparation

Yellow Beeswax	330 g	
Fixed oil	a sufficient quantity	
	To make 1000 g	

Prepare as directed under Ointments, with the above ingredients.

Description Simple Ointment is yellow in color. It has a slight, characteristic odor.

Containers and storage Containers—Tight containers.

Simple Syrup

単シロップ

Simple Syrup is an aqueous solution of Sucrose.

Method of preparation

Sucrose	850 g
Purified Water or Purified	
Water in Containers	a sufficient quantity
	To make 1000 mL

Prepare as directed under Syrups, with the above materials.

Description Simple Syrup is a clear, colorless to pale yellow, viscous liquid. It is odorless and has a sweet taste.

Identification (1) Evaporate Simple Syrup on a water bath to dryness. 1 g of the residue so obtained, when ignited, melts to swell, and decomposes, emitting an odor of caramel, to bulky charcoal.

(2) To 0.1 g of the residue obtained in (1) add 2 mL of dilute sulfuric acid, boil, add 4 mL of sodium hydroxide TS and 3 mL of Fehling's TS, and heat to boiling: a red to dark

red precipitate is produced.

Specific gravity $\langle 2.56 \rangle$ d_{20}^{20} : 1.310 – 1.325

Purity (1) Artificial sweetening agents—To 100 mL of Simple Syrup add 100 mL of water, shake, acidify a 50-mL portion of the solution with dilute sulfuric acid, and make another 50-mL portion alkaline with sodium hydroxide TS. To each portion add 100 mL of diethyl ether, shake, separate the diethyl ether layer, and evaporate the combined diethyl ether extract on a water bath to dryness: the residue has no sweet taste.

(2) Salicylic acid—To the residue obtained in (1) add 2 to 3 drops of dilute iron (III) chloride TS: no purple color develops.

Containers and storage Containers—Tight containers.

Simvastatin

シンバスタチン



 $C_{25}H_{38}O_5$: 418.57 (1*S*,3*R*,7*S*,8*S*,8a*R*)-8-{2-[(2*R*,4*R*)-4-Hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate [79902-63-9]

Simvastatin contains not less than 98.0% and not more than 101.0% of $C_{25}H_{38}O_5$, calculated on the dried basis.

It may contain a suitable antioxidant.

Description Simvastatin occurs as a white, crystalline powder.

It is freely soluble in acetonitrile, in methanol and in ethanol (99.5), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Simvastatin in acetonitrile (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$ and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Simvastatin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Simvastatin as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Simvastatin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $\langle 2.49 \rangle$ [α]_D²⁰: +285 - +300° (50 mg calculated on the dried basis, acetonitrile, 10 mL, 100 mm).

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

Simvastatin in 10 mL of methanol: the solution is clear. Perform the test with this solution as directed under Ultravioletvisible Spectrophotometry <2.24>: the absorbance at 440 nm is not more than 0.10.

(2) Heavy metals <1.07>—To 1.0 g of Simvastatin add 2 mL of sulfuric acid, and heat gently to carbonize. After cooling, add 2 mL of nitric acid and 1 mL of sulfuric acid, heat gently until the white fumes no more evolve, and heat to incinerate at 500 to 600°C. If the incineration is not accomplished, add 0.5 mL of nitric acid, heat in the same manner as above, and ignite at 500 to 600°C to incinerate completely. After cooling, add 2 mL of hydrochloric acid, proceed with this solution according to Method 2, and perform the test. Prepare the control solution by using the same quantities of the same reagents as directed for the preparation of the test solution, and add 2.0 mL of Standard Lead solution and water to make 50 mL (not more than 20 ppm).

(3) Related substances—Dissolve 30 mg of Simvastatin in 20 mL of a mixture of acetonitrile and 0.01 mol/L potassium dihydrogen phosphate TS, pH 4.0 (3:2), and use this solution as the sample solution. Perform the test with $5 \,\mu L$ of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area from the sample solution by the automatic integration method, and calculate the amount of them by the area percentage method: the amounts of the peaks, having the relative retention times of about 0.45, about 0.80, about 2.42, and about 3.80 with respect to simvastatin are not more than 0.2%, respectively; the amount of the peak with a relative retention time of about 2.38 is not more than 0.3%; the amount of the peak with a relative retention time of about 0.60 is not more than 0.4%; and the amount of each peak other than simvastatin and other than the peaks mentioned above is not more than 0.1%. Furthermore, the total amount of the peaks other than simvastatin and other than the peak with 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 A: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile for liquid chromatography (1:1).

Mobile phase B: A solution of phosphoric acid in acetonitrile for liquid chromatography (1 in 1000).

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

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 - 4.5	100	0
4.5 - 4.6	$100 \rightarrow 95$	$0 \rightarrow 5$
4.6 - 8.0	$95 \rightarrow 25$	$5 \rightarrow 75$
8.0 - 11.5	25	75

Flow rate: 3.0 mL per minute.

Time span of measurement: About 5 times as long as the retention time of simvastatin.

System suitability—

Test for required detectability: To 0.5 mL of the sample solution, add a mixture of acetonitrile and 0.01 mol/L potassium dihydrogen phosphate TS, pH 4.0 (3:2), to make 100 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, 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 μ L of this solution is equivalent to 16 to 24% of the peak area of simvastatin from $5 \,\mu L$ of the solution for system suitability test.

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

System repeatability: When the test is repeated 6 times with 5 μ L of the solution for system suitability test under the above conditions, the relative standard deviation of the peak area of simvastatin is not more than 1.0%.

(4) Residual solvent—Being specified separately.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 30 mg each of Simvastatin and Simvastatin RS (previously determine the loss on drying <2.41> under the same conditions as Simvastatin), dissolve each 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 these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly $5\,\mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of simvastatin for each solution.

Amount (mg) of simvastatin ($C_{25}H_{38}O_5$) = $M_S \times A_T/A_S$

 $M_{\rm S}$: 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 4.6 mm in inside diameter and 33 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μ m 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 for liquid chromatography (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 μ L 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.

System repeatability: When the test is repeated 6 times with 5 μ L 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-Tight containers.

Storage-Under nitrogen atmosphere.

Freeze-dried Smallpox Vaccine

乾燥痘そうワクチン

Freeze-dried Smallpox Vaccine is a preparation for injection which is dissolved before use. It contains live vaccinia virus.

It conforms to the requirements of Freeze-dried Smallpox Vaccine in the Minimum Requirements for Biological Products.

Description Freeze-dried Smallpox Vaccine becomes a white to gray, turbid liquid on addition of solvent.

Freeze-dried Smallpox Vaccine Prepared in Cell Culture

乾燥細胞培養痘そうワクチン

Freeze-dried Smallpox Vaccine Prepared in Cell Culture is a preparation for injection which is dissolved before use. It contains live vaccinia virus.

It conforms to the requirements of Freeze-dried Smallpox Vaccine Prepared in Cell Culture in the Minimum Requirements for Biological Products.

Description Freeze-dried Smallpox Vaccine Prepared in Cell Culture becomes a reddish clear liquid on addition of solvent.

Sodium Acetate Hydrate

酢酸ナトリウム水和物

H₃C-CO₂Na • 3H₂O

C₂H₃NaO₂.3H₂O: 136.08 Monosodium acetate trihydrate [*6131-90-4*]

Sodium Acetate Hydrate, when dried, contains not less than 99.5% of sodium acetate ($C_2H_3NaO_2$: 82.03).

Description Sodium Acetate Hydrate occurs as colorless crystals or a white, crystalline powder. It is odorless or has a slight, acetous odor. It has a cool, saline and slightly bitter taste.

It is very soluble in water, freely soluble in acetic acid (100), soluble in ethanol (95), and practically insoluble in diethyl ether.

It is efflorescent in warm, dry air.

Identification A solution of Sodium Acetate Hydrate (1 in 10) responds to the Qualitative Tests $\langle 1.09 \rangle$ for acetate and for sodium salt.

Purity (1) Clarity and color of solution—Dissolve 2.0 g of Sodium Acetate Hydrate in 20 mL of water: the solution is clear and colorless.

(2) Acidity or alkalinity—Dissolve 1.0 g of Sodium Ace-

tate Hydrate in 20 mL of freshly boiled and cooled water, and add 3 drops of phenolphthalein TS: a red color develops. When cooled to 10°C, or 1.0 mL of 0.01 mol/L hydro-chloric acid VS is added after cooling to 10°C, the red color disappears.

(3) Chloride $\langle 1.03 \rangle$ —Perform the test with 1.0 g of Sodium Acetate Hydrate. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(4) Sulfate $\langle 1.14 \rangle$ —Perform the test with 1.0 g of Sodium Acetate Hydrate. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.017%).

(5) Heavy metals <1.07>—Proceed with 2.0 g of Sodium Acetate 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) Calcium and magnesium—Dissolve 4.0 g of Sodium Acetate Hydrate in 25 mL of water, add 6 g of ammonium chloride, 20 mL of ammonia solution (28) and 0.25 mL of a solution of sodium sulfite heptahydrate (1 in 10), and titrate $\langle 2.50 \rangle$ with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the blue color changes to grayish blue (indicator: 0.1 g of methylthymol blue-potassium nitrate indicator): the amount of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS consumed is not more than 0.5 mL.

(7) Arsenic $\langle 1.11 \rangle$ —Prepare the test solution with 1.0 g of Sodium Acetate Hydrate, according to Method 1, and perform the test (not more than 2 ppm).

(8) Potassium permanganate-reducing substance—Dissolve 1.0 g of Sodium Acetate Hydrate in 100 mL of water, add 5 mL of dilute sulfuric acid, boil, add 0.50 mL of 0.002 mol/L potassium permanganate VS, and further boil for 5 minutes: the red color of the solution does not disappear.

Loss on drying $\langle 2.41 \rangle$ 39.0 – 40.5% (1 g, first at 80°C for 2 hours, and then at 130°C for 2 hours).

Assay Weigh accurately about 0.2 g of Sodium Acetate Hydrate, previously dried, dissolve in 50 mL of acetic acid (100), and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS until the color of the solution changes from yellow to green (indicator: 1 mL of *p*-naphtholbenzein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = $8.203 \text{ mg of } C_2H_3NaO_2$

Containers and storage Containers—Tight containers.

Sodium Aurothiomalate

Mixture of C₄H₃AuNa₂O₄S: 390.08 and C₄H₄AuNaO₄S: 368.09 Monogold monosodium monohydrogen (2*RS*)-2sulfidobutane-1,4-dioate Monogold disodium (2*RS*)-2-sulfidobutane-1,4-dioate [*12244-57-4*, Sodium Aurothiomalate]

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 occurs as white to light yellow, powder or granules.

It is very soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

It 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 a solution of calcium nitrate tetrahydrate (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 (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 granules.

(4) The filtrate obtained in (3) responds to the Qualitative Tests $\langle 1.09 \rangle$ for sodium salt.

(5) The filtrate obtained in (3) responds to the Qualitative Tests $\langle 1.09 \rangle$ for sulfate.

pH <2.54> 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 light yellow.

(2) Heavy metals <1.07>—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 $\langle 1.11 \rangle$ —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 sample 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 $2 \mu L$ each of the sample solution and standard solution as directed under Gas Chromatography $\langle 2.02 \rangle$ according to the following conditions, and calculate 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%.

Amount (mg) of ethanol = $Q_T/Q_S \times 6 \times 0.793$

0.793: Density (g/mL) of ethanol (99.5) 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 inside diameter and 3 m in length, packed with porous styrene-divinylbenzene copolymer for gas chromatography (particle diameter: $150 - 180 \mu$ m) (average pore size: 0.0085μ m; $300 - 400 \text{ m}^2/\text{g}$).

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.

System suitability—

System performance: When the procedure is run with $2 \mu L$ 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 $2 \mu L$ 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%.

Water $\langle 2.48 \rangle$ Not more than 5.0% (0.1 g, coulometric titration). Use a water vaporizer (heating temperature: 105°C; heating time: 30 minutes).

Assay Weigh accurately about 25 mg of Sodium Aurothiomalate, and dissolve in 2 mL of aqua regia by heating. After cooling, 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 sample solution. Separately, pipet 5 mL, 10 mL and 15 mL of Standard Gold Solution for atomic absorption spectrophotometry, add water to make exactly 25 mL, and (3), respectively. Perform the test with the sample solution and standard solutions (1), (2) and (3) as directed under Atomic Absorption Spectrophotometry $\langle 2.23 \rangle$ under the following conditions. Determine the amount of gold in the sample solution using the calibration curve obtained from the absorbances of the standard solutions.

Gas: Combustible gas-Acetylene.

Supporting gas—Air. Lamp: Gold hollow-cathode lamp. Wavelength: 242.8 nm.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Sodium Benzoate

安息香酸ナトリウム

CO₂Na

C₇H₅NaO₂: 144.10 Monosodium benzoate [*532-32-1*]

Sodium Benzoate, when dried, contains not less than 99.0% of $C_7H_5NaO_2$.

Description Sodium Benzoate occurs as white granules, crystals or crystalline powder. It is odorless, and has a sweet and saline taste.

It is freely soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification A solution of Sodium Benzoate (1 in 100) responds to the Qualitative Tests $\langle 1.09 \rangle$ for benzoate and the Qualitative Tests $\langle 1.09 \rangle$ (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) Acidity or alkalinity—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 $\langle 1.14 \rangle$ —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 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 0.120%).

(4) Heavy metals $\langle 1.07 \rangle$ —Dissolve 2.0 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 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).

(5) Arsenic $\langle 1.11 \rangle$ —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 using this solution as the test solution (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 portions of diethyl ether. Combine the diethyl ether extracts, and evaporate the diethyl 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—To 0.10 g of Sodium Benzoate add 1 mL of water and 1 mL of resorcinol-sulfuric acid TS, and heat the mixture in an oil bath heated at a temperature between 120° C and 125° C to evaporate the water, then heat the residue for further 90 minutes, cool, and dissolve in 5 mL of water. To 1 mL of the solution add 10 mL of a solution of sodium hydroxide (43 in 500), shake, then examine under light at a wavelength between 470 nm and 490 nm: the green fluorescence of the solution is not more intense than that of the following control solution.

Control solution: Dissolve 61 mg of potassium hydrogen phthalate in water to make exactly 1000 mL. Pipet exactly 1 mL of the solution, add 1 mL of resorcinol-sulfuric acid TS, and proceed as directed above.

Loss on drying $\langle 2.41 \rangle$ Not more than 1.5% (2 g, 110°C, 4 hours).

Assay Weigh accurately about 1.5 g of Sodium Benzoate, previously dried, and transfer to a 300-mL glass-stoppered flask. Dissolve in 25 mL of water, add 75 mL of diethyl ether and 10 drops of bromophenol blue TS, and titrate $\langle 2.50 \rangle$ with 0.5 mol/L hydrochloric acid VS, while mixing the aqueous and diethyl ether layers by vigorous shaking, until a persistent, light green color is produced in the aqueous layer.

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Each mL of 0.5 mol/L hydrochloric acid VS
= 72.05 mg of C<sub>7</sub>H<sub>5</sub>NaO<sub>2</sub>
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Containers and storage Containers-Well-closed containers.

Sodium Bicarbonate

Sodium Hydrogen Carbonate

炭酸水素ナトリウム

NaHCO₃: 84.01

Sodium Bicarbonate contains not less than 99.0% of NaHCO₃.

Description Sodium Bicarbonate occurs as white crystals or crystalline powder. It is odorless, and has a characteristic, saline taste.

It is soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It slowly decomposes in moist air.

Identification A solution of Sodium Bicarbonate (1 in 30) responds to the Qualitative Tests <1.09> for sodium salt and for bicarbonate.

pH <2.54> Dissolve 1.0 g of Sodium Bicarbonate in 20 mL

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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 $\langle 1.03 \rangle$ —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 using this solution as the test solution. Prepare the control solution with 0.45 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.040%).

(3) 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 phenol-phthalein TS: no red color develops immediately.

(4) Ammonium—Heat 1.0 g of Sodium Bicarbonate: the gas evolved does not change moistened red litmus paper to blue.

(5) Heavy metals $\langle 1.07 \rangle$ —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 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 4.5 mL of hydrochloric acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 5 ppm).

(6) Arsenic $\langle 1.11 \rangle$ —Dissolve 1.0 g of Sodium Bicarbonate in 3 mL of water and 2 mL of hydrochloric acid, and perform the test using this solution as the test solution (not more than 2 ppm).

Assay Weigh accurately about 2 g of Sodium Bicarbonate, dissolve in 25 mL of water, and titrate with 0.5 mol/L sulfuric acid VS. When the color of the solution changes from blue to yellow-green, boil with caution, cool, and continue the titration $\langle 2.50 \rangle$ until a greenish yellow color develops (indicator: 2 drops of bromocresol green TS).

Each mL of 0.5 mol/L sulfuric acid VS = 84.01 mg of NaHCO₃

Containers and storage Containers—Tight containers.

Sodium Bicarbonate Injection

炭酸水素ナトリウム注射液

Sodium Bicarbonate Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of sodium hydrogen carbonate (NaHCO₃: 84.01).

Method of preparation Prepare as directed under Injections, with Sodium Bicarbonate.

Description Sodium Bicarbonate Injection is a clear, color-less 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 <1.09> for sodium salt and for bicarbonate.

pH <2.54> 7.0 - 8.5

Bacterial endotoxins <4.01> Less than 5.0 EU/mEq.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of Sodium Bicarbonate Injection, equivalent to about 2 g of sodium hydrogen carbonate (NaHCO₃), titrate with 0.5 mol/L sulfuric acid VS, and proceed as directed in the Assay under Sodium Bicarbonate.

Each mL of 0.5 mol/L sulfuric acid VS = $84.01 \text{ mg of NaHCO}_3$

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Sodium Bisulfite

Sodium Hydrogen Sulfite

亜硫酸水素ナトリウム

NaHSO₃: 104.06

Sodium Bisulfite is a mixture of sodium hydrogensulfite and sodium pyrosulfite.

It contains not less than 64.0% and not more than 67.4% of sulfur dioxide (SO₂: 64.06).

Description Sodium Bisulfite occurs as white granules or powder, having the odor of sulfur dioxide.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

A solution of Sodium Bisulfite (1 in 20) is acid.

It is slowly affected by air or by light.

Identification A solution of Sodium Bisulfite (1 in 20) responds to the Qualitative Tests $\langle 1.09 \rangle$ for sodium salt and for bisulfite.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Sodium Bisulfite in 10 mL of water: the solution is clear and colorless.

(2) Thiosulfate—Dissolve 1.0 g of Sodium Bisulfite 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 $\langle 1.07 \rangle$ —Dissolve 1.0 g of Sodium Bisulfite in 10 mL of water, add 5 mL of hydrochloric acid, and evaporate on a water bath to dryness. To the residue 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 as follows: evaporate 5 mL of hy-

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drochloric acid on a water bath to dryness, and add 2 mL of dilute acetic acid and 2.0 mL of Standard Lead Solution, and dilute with water to make 50 mL (not more than 20 ppm).

(4) Iron $\langle 1.10 \rangle$ —Prepare the test solution with 1.0 g of Sodium Bisulfite 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 $\langle 1.11 \rangle$ —Dissolve 0.5 g of Sodium Bisulfite in 10 mL of water. Add 1 mL of sulfuric acid, heat on a sand bath until white fumes are evolved, add water to make 5 mL, and perform the test with this solution as the test solution (not more than 4 ppm).

Assay Weigh accurately about 0.15 g of Sodium Bisulfite, and transfer immediately into an iodine flask containing exactly 50 mL of 0.05 mol/L iodine VS, stopper, shake, and allow to stand for 5 minutes in a dark place. Add 1 mL of hydrochloric acid, and titrate $\langle 2.50 \rangle$ the excess iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L iodine VS = 3.203 mg of SO_2

Containers and storage Containers—Tight containers.

Storage—Light-resistant, preferably well-filled, and not exceeding $30^{\circ}C$.

Sodium Borate

ホウ砂

Na₂B₄O₇.10H₂O: 381.37

Sodium Borate contains not less than 99.0% and not more than 103.0% of $Na_2B_4O_7.10H_2O$.

Description Sodium Borate occurs as colorless or white crystals or a white, crystalline powder. It is odorless, and has a slightly characteristic, saline taste.

It is freely soluble in glycerin, soluble in water, and practically insoluble in ethanol (95), in ethanol (99.5) and in diethyl 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 $\langle 1.09 \rangle$ for sodium salt and for borate.

pH <2.54> 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 $\langle 1.07 \rangle$ —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 using this solution as the test solution. 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 $\langle 1.11 \rangle$ —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 $\langle 2.50 \rangle$ 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

臭化ナトリウム

NaBr: 102.89

Sodium Bromide, when dried, contains not less than 99.0% of NaBr.

Description Sodium Bromide occurs as colorless or white crystals or crystalline powder. It is odorless.

It is freely soluble in water, and soluble in ethanol (95).

It is hygroscopic, but not deliquescent.

Identification A solution of Sodium Bromide (1 in 10) responds to the Qualitative Tests $\langle 1.09 \rangle$ 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) Alkalinity—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 $\langle 1.14 \rangle$ —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 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 <1.07>—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) 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.

(9) Arsenic $\langle 1.11 \rangle$ —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 $\langle 2.41 \rangle$ Not more than 5.0% (1 g, 110°C, 4 hours).

Assay Weigh accurately about 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 $\langle 2.50 \rangle$ 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.

Each mL of 0.1 mol/L silver nitrate VS = 10.29 mg of NaBr

Containers and storage Containers—Tight containers.

Sodium Carbonate Hydrate

炭酸ナトリウム水和物

Na2CO3.10H2O: 286.14

Sodium Carbonate Hydrate contains not less than 99.0% and not more than 103.0% of $Na_2CO_3.10H_2O$.

Description Sodium Carbonate Hydrate occurs as colorless or white crystals.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

A solution of Sodium Carbonate Hydrate (1 in 10) is alkaline.

It is efflorescent in air.

It liquefies in its water of crystallization at 34° C, and becomes anhydrous at above 100° C.

Identification A solution of Sodium Carbonate Hydrate (1 in 20) responds to the Qualitative Tests $\langle 1.09 \rangle$ for sodium salt and for carbonate.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Sodium Carbonate Hydrate in 5 mL of water: the solution is clear and colorless.

(2) Chloride $\langle 1.03 \rangle$ —Dissolve 0.5 g of Sodium Carbonate Hydrate in 10 mL of water, add 7 mL of dilute nitric acid, dilute with water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.071%).

(3) Heavy metals <1.07>—Dissolve 2.0 g of Sodium Carbonate Hydrate in 10 mL of water, add 8 mL of dilute hydrochloric acid, and evaporate to dryness on a water bath. Dissolve the residue in 35 mL of water and 2 mL of dilute acetic acid, dilute with water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 8 mL of dilute hydrochloric acid and 2.0 mL of Standard Lead Solution, and dilute with water to make 50 mL (not more than 10 ppm).

(4) Arsenic $\langle 1.11 \rangle$ —Prepare the test solution with 0.65 of Sodium Carbonate Hydrate according to Method 1, and perform the test (not more than 3.1 ppm).

Loss on drying <2.41> 61.0~63.0% (1 g, 105°C, 4 hours).

Assay Dissolve about 3 g of Sodium Carbonate Hydrate, weighed accurately, in 25 mL of water, and titrate with 0.5 mol/L sulfuric acid VS until the color of the solution changes from blue to yellow-green. Boil cautiously, cool, and further titrate $\langle 2.50 \rangle$ until a greenish yellow color appears (indicator: 2 drops of bromocresol green TS).

Each mL of 0.5 mol/L sulfuric acid VS = 143.1 mg of Na₂CO₃.10H₂O

Containers and storage Containers—Tight containers.

Dried Sodium Carbonate

乾燥炭酸ナトリウム

Na₂CO₃: 105.99

Dried Sodium Carbonate, when dried, contains not less than 99.0% of Na₂CO₃.

Description Dried Sodium Carbonate occurs as white crystals or crystalline powder.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

A solution of Dried Sodium Carbonate (1 in 10) is alkaline.

It is hygroscopic.

Identification A solution of Dried Sodium Carbonate (1 in 20) responds to the Qualitative Tests $\langle 1.09 \rangle$ for sodium salt and for carbonate.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Dried Sodium Carbonate in 10 mL of water: the solution is clear and colorless.

(2) Chloride $\langle 1.03 \rangle$ —Dissolve 0.5 g of Dried Sodium Carbonate in 10 mL of water, add 12 mL of dilute nitric acid, dilute with water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.071%).

(3) Heavy metals $\langle 1.07 \rangle$ —Dissolve 1.0 g of Dried Sodium Carbonate in 10 mL of water, add 7.5 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 35 mL of water and 2 mL of dilute acetic acid, dilute with water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 7.5 mL of dilute hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid and 2.0 mL of Standard Lead Solution, and dilute with water to make 50 mL (not more than 20 ppm).

(4) Arsenic $\langle 1.11 \rangle$ —Prepare the test solution with 0.65 g of Dried Sodium Carbonate according to Method 1, and perform the test (not more than 3.1 ppm).

Loss on drying $\langle 2.41 \rangle$ Not more than 2.0% (2 g, 106°C, 4 hours).

Assay Dissolve about 1.2 g of Dried Sodium Carbonate,

weighed accurately, in 25 mL of water, and titrate with 0.5 mol/L sulfuric acid VS until the color of the solution changes from blue to yellow-green. Then boil cautiously, cool, and further titrate $\langle 2.50 \rangle$ until a greenish yellow color develops (indicator: 2 drops of bromocresol green TS).

Each mL of 0.5 mol/L sulfuric acid VS = 53.00 mg of Na₂CO₃

Containers and storage Containers—Tight containers.

Sodium Chloride

塩化ナトリウム

NaCl: 58.44

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (\bullet).

Sodium Chloride contains not less than 99.0% and not more than 100.5% of NaCl, calculated on the dried basis.

***Description** Sodium Chloride occurs as colorless or white, crystals or crystalline powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5). \blacklozenge

Identification (1) A solution of Sodium Chloride (1 in 20) responds to the Qualitative Tests $\langle 1.09 \rangle$ for sodium salt.

(2) A solution of Sodium Chloride (1 in 20) responds to the Qualitative Tests $\langle 1.09 \rangle$ 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. \bullet

(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 sample solution. To 20 mL of the sample solution add 0.1 mL of bromothymol 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 sample 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) Sulfates—To 7.5 mL of the sample solution obtained in (2) add water to make 30 mL, and use this solution as the sample solution. Separately, dissolve 0.181 g of potassium sulfate in diluted ethanol (3 in 10) to make exactly 500 mL. Pipet 5 mL of this solution, and add diluted 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 minutes. To 2.5 mL of this solution add 15 mL of the sample solution and 0.5 mL of acetic acid (31), and allow to stand for 5 minutes: any turbidity produced does 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 sample solution.

(4) Phosphates—To 2.0 mL of the sample 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 molybdatesulfuric 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.

(5) Bromides—To 0.50 mL of the sample solution obtained in (2) add 4.0 mL of water, 2.0 mL of dilute phenol red TS and 1.0 mL of a solution of sodium toluenesulfonchloramide trihydrate (1 in 10,000), and mix immediately. After allowing to stand for 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 sample solution. Separately, to 5.0 mL of a solution of potassium bromide (3 in 1,000,000) add 2.0 mL of dilute phenol red TS and 1.0 mL of a solution of sodium toluenesulfonchloramide trihydrate (1 in 10,000), and mix immediately. Proceed in the same manner as for the preparation of the sample solution, and use the solution so obtained as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24> using water as the control: the absorbance at 590 nm of the sample solution is not more than that of the standard solution.

(6) Iodides—Wet 5 g of Sodium Chloride with dropwisely added 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.

(7) 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.

•(8) Heavy metals $\langle 1.07 \rangle$ —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).

(9) Iron—To 10 mL of the sample solution obtained in (2) add 2 mL of a solution of citric acid monohydrate (1 in 5) and 0.1 mL of mercapto acetic acid, alkalize with ammonia TS, add water to make 20 mL, and allow to stand for 5 minutes: the solution has not more color 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 mercapto acetic acid, and proceed in the same manner as directed for the sample solution.

(10) Barium—To 5.0 mL of the sample 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 sample solution obtained in (2) add 7.0 mL of water, and allow to stand for 2 hours.
(11) 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 dihydrogen 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 dihydrogen ethylenediamine tetraacetate VS: the color of the solution is a blue-purple.

•(12) Arsenic $\langle 1.11 \rangle$ —Prepare the test solution with 1.0 g of Sodium Chloride according to Method 1, and perform the test (not more than 2 ppm).

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 2 hours).

Assay Weigh accurately about 50 mg of Sodium Chloride, dissolve in 50 mL of water, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L silver nitrate VS (potentiometric titration).

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% 塩化ナトリウム注射液

10% Sodium Chloride Injection is an aqueous solution for injection.

It 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 or Sterile	Water
for Injection in Containers	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. It has a saline taste.

It is neutral.

Identification 10% Sodium Chloride Injection responds to the Qualitative Tests $\langle 1.09 \rangle$ for sodium salt and for chloride.

Bactetial endotoxins <4.01> Less than 3.6 EU/mL.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter $\langle 6.06 \rangle$ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: 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 $\langle 2.50 \rangle$, with vigorous shaking, with 0.1 mol/L silver nitrate VS (indicator: 3 drops of fluorescein sodium TS).

Each mL 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 Solution

0.9% Sodium Chloride Injection Isotonic Salt Solution Isotonic Sodium Chloride Injection

生理食塩液

Isotonic Sodium Chloride Solution is an aqueous solution for injection.

It contains not less than 0.85 w/v% and not more than 0.95 w/v% of sodium chloride (NaCl: 58.44).

Method of preparation

Sodium Chloride	9 g
Water for Injection or Sterile V	Vater
for Injection in Containers	a sufficient quantity
	To make 1000 mL

Prepare as directed under Injections, with the above ingredients.

No preservative is added.

Description Isotonic Sodium Chloride Solution is a clear, colorless liquid. It has a slightly saline taste.

Identification Isotonic Sodium Chloride Solution responds to the Qualitative Tests <1.09> for sodium salt and for chloride.

pH <2.54> 4.5 - 8.0

Purity (1) Heavy metals $\langle 1.07 \rangle$ —Concentrate 100 mL of Isotonic Sodium Chloride Solution to about 40 mL on 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 $\langle 1.11 \rangle$ —Prepare the test solution with 20 mL of Isotonic Sodium Chloride Solution, and perform the test (not more than 0.1 ppm).

Bacterial endotoxins <4.01> Less than 0.50 EU/mL.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter $\langle 6.06 \rangle$ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly 20 mL of Isotonic Sodium Chloride Solution, add 30 mL of water, and titrate $\langle 2.50 \rangle$ 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 (⁵¹Cr) Injection

クロム酸ナトリウム (⁵¹Cr) 注射液

Sodium Chromate (${}^{51}Cr$) Injection is an aqueous solution for injection.

It contains a chromium-51 (51 Cr) in the form of sodium chromate.

It conforms to the requirements of Sodium Chromate (⁵¹Cr) Injection in the Minimum Requirements for Radiopharmaceuticals.

Test for Extractable Volume of Parentaral Preparations and Insoluble Particulate Matter Test for Injections are not applied to this injection.

Description Sodium Chromate (⁵¹Cr) Injection is a clear, light yellow liquid. It is odorless or has an odor of the preservatives.

Sodium Citrate Hydrate

クエン酸ナトリウム水和物

C₆H₅Na₃O₇.2H₂O: 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 occurs as colorless crystals, or a white, crystalline powder. It is odorless, and has a cooling, saline taste.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

Identification A solution of Sodium Citrate Hydrate (1 in 20) responds to the Qualitative Tests $\langle 1.09 \rangle$ for citrate and for sodium salt.

pH $\langle 2.54 \rangle$ 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 $\langle 1.03 \rangle$ —Take 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 $\langle 1.14 \rangle$ —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 $\langle 1.07 \rangle$ —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 $\langle 1.11 \rangle$ —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 a solution of 1.0 g of Sodium Citrate Hydrate in 2 mL of water add 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 $\langle 1.15 \rangle$ —Take 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 Matching Fluid K.

Loss on drying <2.41> 10.0 – 13.0% (1 g, 180°C, 2 hours).

Assay Weigh accurately about 0.2 g of Sodium Citrate Hydrate, previously dried, add 30 mL of acetic acid for nonaqueous titration, warm to dissolve, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

> Each mL of 0.1 mol/L perchloric acid VS = $8.602 \text{ mg of } C_6H_5Na_3O_7$

Containers and storage Containers—Tight containers.

Sodium Citrate Injection for Transfusion

輸血用クエン酸ナトリウム注射液

Sodium Citrate Injection for Transfusion is an aqueous solution for injection.

It contains not less than 9.5 w/v% and not more than 10.5 w/v% of sodium citrate hydrate (C₆H₅Na₃O₇.2H₂O: 294.10).

Method of preparation

Sodium Citrate Hydrate	100 g
Water for Injection or Sterile	Water
for Injection in Containers	a sufficient quantity
	To make 1000 mL

Prepare as directed under Injections, with the above ingredients.

No preservatives may be added.

Description Sodium Citrate Injection for Transfusion is a clear, colorless liquid.

Identification Sodium Citrate Injection for Transfusion responds to the Qualitative Tests $\langle 1.09 \rangle$ for sodium salt and for citrate.

pH <2.54> 7.0 - 8.5

Bacterial endotoxins <4.01> Less than 5.6 EU/mL.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Pipet 5 mL of Sodium Citrate Injection for Transfusion, and add water to make exactly 25 mL. Evaporate 10 mL of this solution, exactly measured, 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. Cool, titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 9.803 mg of $C_6H_5Na_3O_7.2H_2O$

Containers and storage Containers-Hermetic containers.

Diagnostic Sodium Citrate Solution

診断用クエン酸ナトリウム液

Diagnostic Sodium Citrate Solution contains not less than 3.3 w/v% and not more than 4.3 w/v% of sodium citrate hydrate (C₆H₅Na₃O₇.2H₂O: 294.10).

The requirements as described for aqueous injections under Injections are applicable.

Method of preparation

Sodium Citrate Hydrate	38 g
Water for Injection or Sterile	Water
for Injection in Containers	a sufficient quantity
	To make 1000 mL

Prepare as directed under Injections, with the above ingredients.

No preservative may be added.

Description Diagnostic Sodium Citrate Solution is a clear, colorless liquid.

Identification Diagnostic Sodium Citrate Solution responds to the Qualitative Tests $\langle 1.09 \rangle$ for sodium salt and for citrate.

pH <2.54> 7.0 - 8.5

Assay Pipet 5 mL of Diagnostic Sodium Citrate Solution, 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. Cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

> Each mL of 0.1 mol/L perchloric acid VS = 9.803 mg of C₆H₅Na₃O₇.2H₂O

Containers and storage Containers-Hermetic containers.

Sodium Cromoglicate

クロモグリク酸ナトリウム



 $C_{23}H_{14}Na_2O_{11}$: 512.33 Disodium 5,5'-(2-hydroxypropane-1,3-diyl)bis(oxy)bis(4oxo-4*H*-chromene-2-carboxylate) [*15826-37-6*]

Sodium Cromoglicate contains not less than 98.0% of $C_{23}H_{14}Na_2O_{11}$, calculated on the dried basis.

Description Sodium Cromoglicate occurs as a white, crystalline powder. It is odorless and tasteless at first, and later develops a slightly bitter taste.

It is freely soluble in water, sparingly soluble in propylene glycol, very slightly soluble in ethanol (95), and practically insoluble in 2-propanol and in diethyl ether.

It is hygroscopic.

It gradually acquires 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 produced. After cooling, add 0.5 mL of concentrated diazobenzene sulfonic acid TS: a dark red color is produced.

(2) Determine the absorption spectrum of a solution of Sodium Cromoglicate in phosphate buffer solution, pH 7.4 (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Sodium Cromoglicate responds to the Qualitative Tests <1.09> 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) Acidity or alkalinity—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 sample solution. To 20 mL of the sample solution add 0.25 mL of 0.1 mol/L sodium hydroxide VS: a blue color is produced. To another 20 mL of the sample solution add 0.25 mL of 0.1 mol/L hydrochloric acid VS: a yellow color is produced.

(3) Heavy metals <1.07>—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).

1400 Disodium Edetate Hydrate / Official Monographs

(4) Oxalate—Dissolve 0.25 g of Sodium Cromoglicate in water to make exactly 50 mL, and use this solution as the sample solution. Separately, dissolve 49 mg of oxalic acid dihydrate, 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 sample solution and standard solution, add exactly 5 mL of iron salicylate TS to each solution, and add water to make 50 mL. Determine the absorbances of these solutions as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$ using water as the blank: the absorbance of the sample 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 sample solution. Pipet 1 mL of the sample 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 these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 10 μ L each of the sample 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, 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 sample solution is not more intense than the spot from the standard solution.

Loss on drying $\langle 2.41 \rangle$ 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 $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid-1,4-ioxane VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid-1,4-dioxane VS = $25.62 \text{ mg of } C_{23}H_{14}Na_2O_{11}$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Disodium Edetate Hydrate

EDTA Sodium Hydrate

エデト酸ナトリウム水和物



 $C_{10}H_{14}N_2Na_2O_8.2H_2O: 372.24$

Disodium dihydrogen ethylenediaminetetraacetate dihydrate [6381-92-6]

Disodium Edetate Hydrate contains not less than 99.0% of $C_{10}H_{14}N_2Na_2O_8.2H_2O$.

Description Disodium Edetate Hydrate occurs as white crystals or crystalline powder. It is odorless and has a slight, acid taste.

It is soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

Identification (1) Dissolve 0.01 g of Disodium Edetate Hydrate in 5 mL of water, add 2 mL of a solution of potassium chromate (1 in 200) and 2 mL of arsenic (III) trioxide TS, and heat in a water bath for 2 minutes: a purple color develops.

(2) Dissolve 0.5 g of Disodium Edetate Hydrate in 20 mL of water, and add 1 mL of dilute hydrochloric acid: a white precipitate is produced. Collect the precipitate, wash with 50 mL of water, and dry at 105°C for 1 hour: the precipitate melts $\langle 2.60 \rangle$ between 240°C and 244°C (with decomposition).

(3) A solution of Disodium Edetate Hydrate (1 in 20) responds to the Qualitative Tests $\langle 1.09 \rangle$ (1) for sodium salt.

pH $\langle 2.54 \rangle$ Dissolve 1 g of Disodium Edetate Hydrate in 100 mL of water: the pH of this solution is between 4.3 and 4.7.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Disodium Edetate Hydrate in 50 mL of water: the solution is clear and colorless.

(2) Cyanide—Transfer 1.0 g of Disodium Edetate Hydrate to a round-bottomed flask, dissolve in 100 mL of water, add 10 mL of phosphoric acid, and distil. Place 15 mL of 0.5 mol/L sodium hydroxide VS in a 100-mL measuring cylinder, which is used as a receiver, and immerse the bottom end of the condenser into the solution. Distil the mixture until the distillate measures 100 mL, and use this solution as the sample solution. Transfer 20 mL of the sample solution to a glass-stoppered test tube, add 1 drop of phenolphthalein TS, neutralize with dilute acetic acid, and add 5 mL of phosphate buffer solution, pH 6.8, and 1.0 mL of diluted sodium toluenesulfonchloramide TS (1 in 5). Immediately stopper the tube, mix gently, and allow to stand for a few minutes. Mix well with 5 mL of pyridine-pyrazolone TS, and allow to stand between 20°C and 30°C for 50 minutes: the solution has no more color than the following control solution.

Control solution: Pipet 1.0 mL of Standard Cyanide Solution, add 15 mL of 0.5 mol/L sodium hydroxide VS and water to make exactly 1000 mL, transfer 20 mL of this solution to a glass-stoppered test tube, and proceed as directed for the sample solution.

(3) Heavy metals <1.07>—Proceed with 2.0 g of Disodium Edetate 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 $\langle 1.11 \rangle$ —Prepare the test solution with 1.0 g of Disodium Edetate Hydrate according to Method 1, and perform the test (not more than 2 ppm).

Residue on ignition <2.44> 37.0 – 39.0% (1 g).

Assay Weigh accurately about 1 g of Disodium Edetate Hydrate, dissolve in 50 mL of water, add 2 mL of ammoniaammonium chloride buffer solution, pH 10.7, and 0.04 g of eriochrome black T-sodium chloride indicator, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L zinc VS until the color of the solution changes from blue to red. Each mL of 0.1 mol/L zinc VS = 37.22 mg of C₁₀H₁₄N₂Na₂O₈.2H₂O

Containers and storage Containers-Well-closed containers.

Sodium Fusidate

フシジン酸ナトリウム



C31H47NaO6: 538.69

Monosodium (17Z)-*ent*-16 α -acetoxy-3 β ,11 β -dihydroxy-4 β ,8 β ,14 α -trimethyl-18-nor-5 β ,10 α -cholesta-17(20),24dien-21-oate [751-94-0]

Sodium Fusidate is the sodium salt of a substance having antibacterial activity produced by the growth of *Fusidium coccineum*.

It contains not less than $935 \,\mu g$ (potency) and not more than $969 \,\mu g$ (potency) per mg, calculated on the anhydrous basis. The potency of Sodium Fusidate is expressed as mass (potency) of fusidic acid (C₃₁H₄₈O₆: 516.71).

Description Sodium Fusidate occurs as white, crystals of crystalline powder.

It is freely soluble in water, in methanol and in ethanol (99.5).

Identification (1) Determine the infrared absorption spectra of Sodium Fusidate as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Sodium Fusidate responds to the Qualitative Tests <1.09> (1) for sodium salt.

Purity Heavy metals $\langle 1.07 \rangle$ —Proceed with 2.0 g of Sodium Fusidate 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).

Water $\langle 2.48 \rangle$ Not more than 2.0% (1 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics $\langle 4.02 \rangle$ according to the following conditions.

(i) Test organism—Staphylococcus aureus ATCC 6538 P

(ii) Culture medium—Use the medium ii in 3) Medium for other organisms under (1) Agar media for seed and base layer.

(iii) Standard solutions-Weigh accurately an amount of

Diethanolamine Fusidate RS, equivalent to about 20 mg (potency), dissolve in 2 mL of ethanol (95), add water to make exactly 20 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. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 4 μ g (potency) and 1 μ g (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Sodium Fusidate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 20 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains $4 \mu g$ (potency) and $1 \mu g$ (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers. Storage—Light-resistant, and at a temperature 2 to 8°C.

Purified Sodium Hyaluronate

精製ヒアルロン酸ナトリウム



(C₁₄H₂₀NNaO₁₁)_n [9067-32-7]

Purified Sodium Hyaluronate is the sodium salt of glycosaminoglycans composed of disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine obtained from cockscomb or microorganisms.

It contains not less than 90.0% and not more than 105.5% of sodium hyaluronate $(C_{14}H_{20}NNaO_{11})_n$, calculated on the dried basis.

It is composed of an average molecular mass of the sodium salt of hyaluronic acid between 500,000 and 1,200,000 or between 1,500,000 and 3,900,000.

The average molecular mass of Purified Sodium Hyaluronate should be labeled.

Description Purified Sodium Hyaluronate occurs as white powder, granules or fibrous masses.

It is sparingly soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

Identification (1) Determine the infrared absorption spectrum of Purified Sodium Hyaluronate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Purified Sodium Hyaluronate (1 in 1000) responds to the Qualitative Tests $\langle 1.09 \rangle$ (1) for sodium salt.

Viscosity <2.53> Weigh accurately an amount of Purified Sodium Hyaluronate so that the downflowing time of its solution in 100 mL of 0.2 mol/L sodium chloride TS is 2.0 to 2.4 times longer than that of 0.2 mol/L sodium chloride TS, dissolve in 0.2 mol/L sodium chloride TS to make exactly 100 mL, and use this solution as the sample solution (1). Pipet 16 mL, 12 mL and 8 mL of the sample solution (1), to each add 0.2 mol/L sodium chloride TS to make exactly 20 mL, and use these solutions as the sample solutions (2), (3) and (4), respectively. Perform the test with the sample solutions (1), (2), (3) and (4) as directed under Method 1 at 30 \pm 0.1°C using a Ubbelohde-type viscometer in which the downflowing time for 0.2 mol/L sodium chloride TS is 200 to 300 seconds: the intrinsic viscosity calculated on the dried basis is between 10.0 and 19.5 dL/g or between 25.0 and 55.0 dL/g.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Purified Sodium Hyaluronate in 10 mL of water: the solution is clear and colorless.

(2) Chloride $\langle 1.03 \rangle$ —Dissolve 0.20 g of Purified Sodium Hyaluronate in 15 mL of water, add 6 mL of dilute nitric acid, and heat on a water bath for 30 minutes. After cooling, add water to make 50 mL. Perform the test using 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 0.124%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Purified Sodium Hyaluronate 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) Residual solvent Being specified separately.

(5) Protein—Weigh accurately about 20 mg of Purified Sodium Hyaluronate, calculated on the dried basis, dissolve in 1.0 mL of dilute sodium hydroxide TS, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of bovine serum albumin, dissolve in dilute sodium hydroxide TS to make exactly 1000 mL, and use this solution as the standard solution. To 1.0 mL each of the sample solution and standard solution add 5.0 mL of alkaline copper TS (2), immediately stir, allow to stand at room temperature for 10 minutes, add 0.5 mL of diluted Folin's TS (1 in 2), immediately stir, and allow to stand at room temperature for 30 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared with 1.0 mL of dilute sodium hydrochloride in the same manner, as the blank: the absorbance of the sample solution at 750 nm does not exceed the absorbance of the standard solution (not more than 0.05%).

(6) Nucleic acid—Determine the absorbance of a solution of 0.10 g Purified Sodium Hyaluronate in 50 mL of water as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, using water as the blank: the absorbance at 260 nm is not more than 0.02.

(7) Other acidic mucopolysaccharides—(In the case of chicken-derived samples) Dissolve 0.25 g of Purified Sodium Hyaluronate in 100 mL of water, and use this solution as the sample solution. Immerse a cellulose acetate membrane 6 cm in length in 0.2 mol/L pirydine-formic acid buffer solution,

pH 3.0. Take out the membrane and remove excessive buffer solution using a filter paper. Place the membrane in an electrophoresis vessel saturated with 0.2 mol/L pyridine-formic acid buffer solution, pH 3.0, and run at 0.5 mA/cm for 1 minute. Apply 2 μ L of the sample solution to the membrane in an area 1 cm in width at 1.5 cm from the anode. Carry out electrophoresis at 0.5 mA/cm for 1 hour. After the electrophoresis, stain the membrane by immersing it in alcian blue staining solution for 10 to 20 minutes. After staining, decolorize sufficiently with diluted acetic acid (100) (3 in 100): no bands other than the principal band appears.

(8) Hemolytic streptococci—(In the case of microorganism-derived samples) Dissolve 0.5 g of Purified Sodium Hyaluronate in sterile isotonic sodium chloride solution to make exactly 100 mL. Take 0.5 mL of this solution, apply to 2 blood agar plates, respectively, using a Conradi stick, and incubate at 37°C for 48 hours: no hemolytic colonies appear, or if any, no streptococci are observed in the colony under a microscope.

(9) Hemolysis—(In the case of microorganism-derived samples) Dissolve 0.40 g of Purified Sodium Hyaluronate in sterile isotonic sodium chloride solution to make exactly 100 mL. To 0.5 mL of this solution add 0.5 mL of 1% blood suspension, mix, allow to stand at 37° C for 2 hours, and, if necessary, centrifuge at 3000 revolutions per minute for 10 minutes: the erythrocytes precipitate and the supernatant liquid is clear as in a blank determination performed in the same manner using 0.5 mL of sterile isotonic sodium chloride solution as the blank and 0.5 mL of sterile purified water as the positive control.

Loss on drying $\langle 2.41 \rangle$ Not more than 15.0% (0.1 g, reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 60°C, 5 hours).

Microbial limit $\langle 4.05 \rangle$ The acceptance criteria of TAMC and TYMC are 10² CFU/g and 10¹ CFU/g.

Average molecular mass (1) In the case of the labeled average molecular mass of between 500,000 and 1,200,000.

Calculate the average molecular mass of Purified Sodium Hyaluronate according to the following equation: it is between 500,000 and 1,200,000. For $[\eta]$, use the maximum viscosity under Viscosity.

Average molecular mass =
$$\left(\frac{[\eta] \times 10^5}{36}\right)^{\frac{1}{0.76}}$$

(2) In the case of the labeled average molecular mass of between 1,500,000 and 3,900,000.

Calculate the average molecular mass of Purified Sodium Hyaluronate according to the following equation: it is between 1,500,000 and 3,900,000. For $[\eta]$, use the maximum viscosity under Viscosity.

Average molecular mass =
$$\left(\frac{[\eta] \times 10^5}{22.8}\right)^{\frac{1}{0.816}}$$

Assay Weigh accurately about 50 mg of Purified Sodium Hyaluronate, and dissolve in water to make exactly 50 mL. Pipet 1 mL of this solution, add water to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of D-Glucuronolactone RS, previously dried (under reduced pressure not exceeding 0.67 kPa, silica gel, 24 hours), and dissolve in 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. Pipet 1 mL each of the sample solution and standard solution, gently add into the 5.0 mL of sodium tetraborate-sulfuric acid TS, previously cooled in ice water, stir while cooling, heat in a water bath for 10 minutes, and cool in ice water. To each solution add exactly 0.2 mL of carbazole TS, stir well, heat in a water bath for 15 minutes, and cool in ice water to room temperature. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 530 nm as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, using a solution, prepared with 1 mL of water in the same manner, as the blank.

Amount (mg) of sodium hyaluronate $[(C_{14}H_{20}NNaO_{11})_n]$ = $M_S \times A_T/A_S \times 2.279$

 $M_{\rm S}$: Amount (mg) of D-Glucuronolactone RS

Containers and storage Containers—Tight containers. Storage—Light-resistant, at not exceeding 15°C.

Sodium Hydroxide

水酸化ナトリウム

NaOH: 40.00

Sodium Hydroxide contains not less than 95.0% of NaOH.

Description Sodium Hydroxide occurs as white fused masses, in small pellets, in flakes, in sticks, and in other forms. It is hard and brittle, and shows a crystalline fracture.

It is freely soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

It rapidly absorbs carbon dioxide in air.

It deliquesces in moist air.

Identification (1) A solution of Sodium Hydroxide (1 in 500) is alkaline.

(2) A solution of Sodium Hydroxide (1 in 25) responds to the Qualitative Tests $\langle 1.09 \rangle$ for sodium salt.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Sodium Hydroxide in 20 mL of water: the solution is clear and colorless.

(2) Chloride $\langle 1.03 \rangle$ —Dissolve 2.0 g of Sodium Hydroxide in water, and add water to make 100 mL. To 25 mL of the solution add 10 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 0.7 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.050%).

(3) Heavy metals $\langle 1.07 \rangle$ —Dissolve 1.0 g of Sodium Hydroxide in 5 mL of water, add 11 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 35 mL of water, add 2 mL of dilute acetic acid and 1 drop of ammonia TS, add water to make 50 mL, and perform the test using this solution as the test solution. Evaporate 11 mL of dilute hydrochloric acid on a water bath to dryness, dissolve the residue in 2 mL of dilute acetic acid and 3.0 mL of Standard Lead Solution, add water to make 50 mL, and use this solution as the control solution (not more than 30 ppm).

(4) Potassium—Dissolve 0.10 g of Sodium Hydroxide in

water and dilute with water to make 40 mL. Add 1.0 mL of dilute acetic acid to 4.0 mL of this solution, and shake. Add 5.0 mL of a solution of sodium tetraphenylboron (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, and dilute with water to make 1000 mL. Add 1.0 mL of dilute acetic acid to 4.0 mL of this solution, shake, and proceed as directed above.

(5) Sodium carbonate—The amount of sodium carbonate (Na₂CO₃: 105.99) is not more than 2.0%, when calculated by the following equation using B (mL) which is obtained in the Assay.

Amount (mg) of sodium carbonate = $105.99 \times B$

(6) Mercury-Dissolve 2.0 g of Sodium Hydroxide in 1 mL of a solution of potassium permanganate (3 in 50) and 30 mL of water, neutralize gradually with purified hydrochloric acid, and add 5 mL of diluted sulfuric acid (1 in 2). To this solution add a solution of hydroxylammonium chloride (1 in 5) until the precipitate of manganese dioxide disappears, add water to make exactly 100 mL, and use this solution as the sample solution. Perform the tests according to the Atomic Absorption Spectrophotometry <2.23> (Cold vapor type) with the sample solution. Place the sample solution in the sample bottle of an atomic absorption spectrophotometer, add 10 mL of tin (II) chloride-sulfuric acid TS, connect the bottle immediately to the atomic absorption spectrophotometer, and circulate air. Read the absorbance $A_{\rm T}$ of the sample solution when the indication of the recorder rises rapidly and becomes constant at the wavelength of 253.7 nm. On the other hand, to 2.0 mL of Standard Mercury Solution add 1 mL of a solution of potassium permanganate (3 in 50), 30 mL of water and a volume of purified hydrochloric acid equal to that used in the preparation of the sample solution, and read the absorbance $A_{\rm S}$ of the solution obtained by the same procedure as used for the sample solution: $A_{\rm T}$ is smaller than $A_{\rm S}$.

Assay Weigh accurately about 1.5 g of Sodium Hydroxide, and dissolve in 40 mL of freshly boiled and cooled water. Cool the solution to 15°C, add 2 drops of phenolphthalein TS, and titrate $\langle 2.50 \rangle$ with 0.5 mol/L sulfuric acid VS until the red color of the solution disappears. Record the amount, A (mL), of 0.5 mol/L sulfuric acid VS consumed. Then add 2 drops of methyl orange TS to the solution, and further titrate $\langle 2.50 \rangle$ with 0.5 mol/L sulfuric acid VS until the solution shows a persistent light red color. Record the amount, B(mL), of 0.5 mol/L sulfuric acid VS consumed. Calculate the amount of NaOH from the difference, A (mL) – B(mL).

> Each mL of 0.5 mol/L sulfuric acid VS = 40.00 mg of NaOH

Containers and storage Containers—Tight containers.

Sodium Iodide

ヨウ化ナトリウム

NaI: 149.89

Sodium Iodide, when dried, contains not less than 99.0% of NaI.

Description Sodium Iodide occurs as colorless crystals or a white, crystalline powder. It is odorless.

It is very soluble in water, and freely soluble in glycerin and in ethanol (95).

It deliquesces in moist air.

Identification A solution of Sodium Iodide (1 in 20) responds to the Qualitative Tests $\langle 1.09 \rangle$ 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) Alkalinity—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 produced.

(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 appears. 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 40-mL 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 develops.

(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 develops immediately.

(7) Heavy metals <1.07>—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 $\langle 1.11 \rangle$ —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 $\langle 2.41 \rangle$ 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 $\langle 2.50 \rangle$ 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 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 = 14.99 mg of NaI

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Sodium Iodide (¹²³I) Capsules

ヨウ化ナトリウム (¹²³I) カプセル

Sodium Iodide (¹²³I) Capsules contain iodine-123 in the form of sodium iodide.

It conforms to the requirements of Sodium Iodide (¹²³I) Capsules in the Minimum Requirements for Radiopharmaceuticals.

Sodium Iodide (¹³¹I) Capsules

ヨウ化ナトリウム (¹³¹I) カプセル

Sodium Iodide (¹³¹I) Capsules contain iodine-131 in the form of sodium iodide.

It conforms to the requirements of Sodium Iodide (¹³¹I) Capsules in the Minimum Requirements for Radiopharmaceuticals.

Sodium Iodide (¹³¹I) Solution

ヨウ化ナトリウム (131]) 液

Sodium Iodide (¹³¹I) Solution contains iodine-131 (¹³¹I) in the form of sodium iodide.

It conforms to the requirements of Sodium Iodide (¹³¹I) Solution in the Minimum Requirements for Radiopharmaceuticals.

Description Sodium Iodide (¹³¹I) Solution is a clear, colorless liquid. It is odorless, or has an odor due to the preservatives or stabilizers.

Sodium Iodohippurate (¹³¹I) Injection

ヨウ化ヒプル酸ナトリウム (1311) 注射液

Sodium Iodohippurate (131 I) Injection is an aqueous solution for injection containing iodine-131 (131 I) in the form of sodium *o*-iodohippurate.

It conforms to the requirements of Sodium Iodohippurate (¹³¹I) Injection in the Minimum Requirements for Radiopharmaceuticals.

Test for Extractable Volume of Parenteral Preparations and Insoluble Particulate Matter Test for Injections are not applied to this injection.

Description Sodium Iodohippurate (¹³¹I) Injection is a clear, colorless liquid. It is odorless or has an odor of the preservatives or stabilizers.

Sodium Iotalamate Injection

イオタラム酸ナトリウム注射液

Sodium Iotalamate Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of iotalamic acid ($C_{11}H_9I_3N_2O_4$: 613.91).

Method of preparation

(1)		
Iotalamic Acid	645 g	
Sodium Hydroxide	42 g	
Water for Injection or Sterile Wa	ater	
for Injection in Containers	a sufficient quantity	
	To make 1000 mL	
(2)		
Iotalamic Acid	772.5 g	
Sodium Hydroxide	50.5 g	
Water for Injection or Sterile Wa	ater	
for Injection in Containers	a sufficient quantity	
	To make 1000 mL	

Prepare as directed under Injections, with the above ingredients (1) or (2).

Description Sodium Iotalamate Injection is a clear, color-less or pale yellow, slightly viscous liquid.

It is gradually colored by light.

Identification (1) To a volume of Sodium Iotalamate Injection, equivalent to 1 g of Iotalamic Acid according to the labeled amount, add 25 mL of water, and add 2.5 mL of dilute hydrochloric acid with thorough stirring: a white precipitate is produced. Filter the precipitate by suction through a glass filter (G4), wash the precipitate with two 10-mL portions of water, and dry at 105°C for 1 hour. Proceed with the precipitate as directed in the Identification (2) under Iotalamic Acid.

(2) Sodium Iotalamate Injection responds to the Qualita-

tive Tests <1.09> (1) for sodium salt.

pH <2.54> 6.5 – 7.7

Purity (1) Primary aromatic amines—To a volume of Sodium Iotalamate Injection, equivalent to 0.20 g of Iotalamic Acid according to the labeled amount, add 15 mL of water, shake, add 4 mL of a solution of sodium nitrite (1 in 100) under ice-cooling, and proceed as directed in the Purity (2) under Iotalamic Acid: the absorbance is not more than 0.17.

(2) Iodine and iodide—To a volume of Sodium Iotalamate Injection, equivalent to 1.5 g of Iotalamic Acid according to the labeled amount, add 20 mL of water and 5 mL of dilute sulfuric acid, shake well, and filter the precipitate by suction through a glass filter (G4). To the filtrate add 5 mL of toluene, and shake vigorously: the toluene layer is colorless. Then add 2 mL of a solution of sodium nitrite (1 in 100), and shake vigorously: the toluene layer has no more color than the following control solution.

Control solution: Dissolve 0.25 g of potassium iodide in water to make 1000 mL. To 2.0 mL of this solution add 20 mL of water, 5 mL of dilute sulfuric acid, 5 mL of toluene and 2 mL of a solution of sodium nitrite (1 in 100), and shake vigorously.

Bacterial endotoxins <4.01> Less than 3.4 EU/mL.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter $\langle 6.06 \rangle$ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Direct inoculation method: it meets the requirement.

Assay Pipet a volume of Sodium Iotalamate Injection, equivalent to about 4 g of iotalamic acid (C₁₁H₉I₃N₂O₄), add water to make exactly 200 mL. Pipet 2 mL of this solution, add water to make exactly 200 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of iotalamic acid for assay, previously dried at 105°C for 4 hours, dissolve in 100 mL of water and 1 mL of sodium hydroxide TS, and add water to make exactly 200 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with $10 \,\mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of iotalamic acid to that of the internal standard.

Amount (mg) of iotalamic acid ($C_{11}H_9I_3N_2O_4$) = $M_S \times Q_T/Q_S$

 $M_{\rm S}$: Amount (mg) of iotalamic acid for assay

Internal standard solution—A solution of L-tryptophan in the mobile phase (3 in 2500).

Operating conditions—

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

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

Column temperature: A constant temperature of about 20° C.

Mobile phase: To 3.9 g of phosphoric acid and 2.8 mL of triethylamine add water to make 2000 mL. To this solution add 100 mL of acetonitrile.

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

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, iotalamic acid 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 $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of iotalamic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Sodium L-Lactate Solution

L-乳酸ナトリウム液

Sodium L-Lactate Solution is an aqueous solution of sodium salt of L-lactic acid.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of sodium L-lactate (C₃H₅NaO₃).

The label states the content amount of sodium L-lactate.

Description Sodium L-Lactate Solution occurs as a clear and colorless viscous liquid. It has no odor or has a slight characteristic odor, and has a slight saline taste.

It is miscible with water or with ethanol (99.5).

Identification To an amount of Sodium L-Lactate Solution, equivalent to 1 g of sodium L-lactate $(C_3H_5NaO_3)$ according to the labeled amount, add water to make 50 mL. This solution responds to the Qualitative Tests $\langle 1.09 \rangle$ for sodium salt and for lactate.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_D^{20}$: $-38 - -44^{\circ}$ To an exact amount of Sodium L-Lactate Solution, equivalent to 2.5 g of sodium L-lactate (C₃H₅NaO₃) according to the labeled amount, add 30 mL of water and 5.0 g of hexaammonium heptamolybdate tetrahydrate, then add water to make exactly 50 mL, and determine using a 100-mm cell.

pH $\langle 2.54 \rangle$ To an amount of Sodium L-Lactate Solution, equivalent to 5 g of sodium L-lactate (C₃H₅NaO₃) according to the labeled amount, add water to make 50 mL: the pH of this solution is between 6.5 and 7.5.

Purity (1) Chloride $\langle 1.03 \rangle$ —Perform the test with an amount of Sodium L-Lactate Solution, equivalent to 1.0 g of sodium L-lactate (C₃H₃NaO₃) according to the labeled

amount. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(2) Sulfate $\langle 1.14 \rangle$ —To an amount of Sodium L-Lactate Solution, equivalent to 2.0 g of sodium L-lactate (C₃H₅NaO₃) according to the labeled amount, add 7 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 0.010%).

(3) Heavy metals $\langle 1.07 \rangle$ —To an amount of Sodium L-Lactate Solution, equivalent to 2.0 g of sodium L-lactate (C₃H₅NaO₃) according to the labeled amount, add 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 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(4) Iron $\langle 1.10 \rangle$ —Prepare the test solution with an amount of Sodium L-Lactate Solution, equivalent to 2.0 g of sodium L-lactate (C₃H₃NaO₃) according to the labeled amount, according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 5 ppm).

(5) Arsenic $\langle 1.11 \rangle$ —To an amount of Sodium L-Lactate Solution, equivalent to 2.5 g of sodium L-lactate (C₃H₅NaO₃) according to the labeled amount, and add water to make 10 mL. Perform the test using 2 mL of this solution as the test solution (not more than 4 ppm).

(6) Sugars—To an amount of Sodium L-Lactate Solution, equivalent to 1.0 g of sodium L-lactate ($C_3H_5NaO_3$) according to the labeled amount, add 10 mL of water and 10 mL of Fehling's TS, and boil for 5 minutes: no red precipitate is produced.

(7) Citric, oxalic, phosphoric and L-tartaric acids—To an amount of Sodium L-Lactate Solution, equivalent to 1.0 g of sodium L-lactate $(C_3H_5NaO_3)$ according to the labeled amount, add 1 mL of water and 1 mL of dilute hydrochloric acid, then add 40 mL of calcium hydroxide TS, and boil for 2 minutes: the solution is not changed.

(8) Volatile fatty acids—To an amount of Sodium L-Lactate Solution, equivalent to 3.0 g of sodium L-lactate (C₃H₅NaO₃) according to the labeled amount, add 2 mL of dilute sulfuric acid, and heat on a water bath: no acetic acid like nor lactic acid like odor is produced.

(9) Cyanide—Transfer an amount of Sodium L-Lactate Solution, equivalent to 1.0 g of sodium L-lactate (C₃H₅NaO₃) according to the labeled amount, to a Nessler tube, add 10 mL of water and 1 drop of phenolphthalein TS, then add dropwise a solution of sodium hydroxide (1 in 10) while shaking until a pale red color appears. Add further 1.5 mL of a solution of sodium hydroxide (1 in 10) and water to make 20 mL, and heat in a water bath for 10 minutes. After cooling, add dropwise dilute hydrochloric acid until a red color of the solution disappears, then add 1 drop of acetic acid (31), 10 mL of phosphate buffer solution, pH 6.8, and 0.25 mL of sodium toluenesulfonchloramide TS, stopper immediately, mix gently, and allow to stand for 5 minutes. Add 15 mL of pyridine-pyrazolone TS and water to make 50 mL, and allow to stand at 25°C for 30 minutes: the color of the solution is not more intense than that of the following control solution.

Control solution: To 1.0 mL of Standard Cyanide Solution add water to make 20 mL. Transfer 1.0 mL of this solu-

tion to a Nessler tube, add 10 mL of water and 1 drop of phenolphthalein TS, then proceed in the same manner as descried above.

(10) Methanol-Transfer an amount of Sodium L-Lactate Solution, equivalent to 5.0 g of sodium L-lactate (C₃H₅NaO₃) according to the labeled amount, to a distilling flask of the apparatus for alcohol number determination <1.01>, add 10 mL of water, and distill. Pipet 5 mL of the distillate, add water to make exactly 10 mL, and use this solution as the sample solution. Separately, to exactly 1.0 mL of methanol add water to make exactly 100 mL. Pipe 5 mL of this solution, add water to make exactly 200 mL. Pipet 5 mL of this solution, add water to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions: the peak area of methanol obtained from the sample solution is not larger than that from the standard solution (not more than 0.025%). **Operating** conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 1.5 m in length, packed with porous ethyl vinylbenzene-divinylbenzene copolymer for gas chromatography (149 – 177 μ m in particle diameter).

Column temperature: A constant temperature of about $120^{\circ}C$.

Injection port and detector temperature: A constant temperature of about 125°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of methanol is about 2 minutes.

System suitability-

System performance: To 1 mL of methanol and 1 mL of ethanol (99.5) add water to make 100 mL. To 5 mL of this solution add water to make 200 mL. To 5 mL of this solution add water to make 10 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, methanol and ethanol 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 $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methanol is not more than 5%.

Assay Weigh accurately an amount of Sodium L-Lactate Solution, equivalent to about 0.25 g of sodium L-lactate $(C_3H_5NaO_3)$, dry at 105°C for 4 hours, add 50 mL of acetic acid (100), and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS until the color of solution changes from purple to yellow-green through blue-green (indicator: 2 drops of crystal violet TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = $11.21 \text{ mg of } C_3H_5NaO_3$

Containers and storage Containers—Tight containers.

Sodium Lauryl Sulfate

ラウリル硫酸ナトリウム

Sodium Lauryl Sulfate is a mixture of sodium alkyl sulfate consisting chiefly of sodium lauryl sulfate ($C_{12}H_{25}NaO_4S$: 288.38).

Description Sodium Lauryl Sulfate occurs as white to light yellow crystals or powder. It has a slightly characteristic odor.

It is sparingly soluble in methanol and in ethanol (95).

A solution of Sodium Lauryl Sulfate (1 in 10) is a clear or an opalescent solution, which foams on agitation.

Identification (1) To 0.2 g of the residue obtained in Total alcohol content add 4 mL of bromine-cyclohexane TS with vigorous shaking, add 0.3 g of *N*-bromosuccinimide, and heat in a water bath at 80°C for 5 minutes: a red color develops.

(2) A solution of Sodium Lauryl Sulfate (1 in 10) responds to the Qualitative Tests $\langle 1.09 \rangle$ (1) for sodium salt.

(3) To a solution of Sodium Lauryl Sulfate (1 in 10) add dilute hydrochloric acid to make acid, boil gently, and cool: the solution responds to the Qualitative Tests $\langle 1.09 \rangle$ for sulfate.

Purity (1) Alkalinity—Dissolve 1.0 g of Sodium Lauryl Sulfate in 100 mL of water, add 2 drops of phenol red TS and 0.60 mL of 0.1 mol/L hydrochloric acid VS: the solution remains yellow.

(2) Sodium chloride—Dissolve about 5 g of Sodium Lauryl Sulfate, accurately weighed, in 50 mL of water, neutralize the solution with dilute nitric acid, if necessary, add exactly 5 mL of 0.1 mol/L sodium chloride TS, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L silver nitrate VS (indicator: 2 drops of fluorescein sodium TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS = 5.844 mg of NaCl

The combined content of sodium chloride (NaCl: 58.44) and sodium sulfate (Na₂SO₄: 142.04) obtained in the next paragraph (3) is not more than 8.0%.

(3) Sodium sulfate—Dissolve about 1 g of Sodium Lauryl Sulfate, accurately weighed, in 10 mL of water, add 100 mL of ethanol (95), and heat at a temperature just below the boiling point for 2 hours. Filter through a glass filter (G4) while hot, and wash with 100 mL of boiling ethanol (95). Dissolve the precipitate by washing with 150 mL of water, collecting the washings in a beaker. Add 10 mL of hydrochloric acid, heat to boiling, add 25 mL of barium chloride TS, and allow to stand overnight. Collect the precipitate, and wash with water until the last washing shows no opalescence with silver nitrate TS. Dry the precipitate, ignite to a constant mass between 500°C and 600°C by raising the temperature gradually, and weigh as barium sulfate (BaSO₄: 233.39).

Amount (mg) of sodium sulfate (Na₂SO₄) = amount (mg) of barium sulfate (BaSO₄) \times 0.609

(4) Unsulfated alcohols—Dissolve about 10 g of Sodium Lauryl Sulfate, accurately weighed, in 100 mL of water, add

100 mL of ethanol (95), and transfer to a separator. Extract the solution with three 50-mL portions of petroleum benzin. If an emulsion forms, sodium chloride may be added to promote separation of the two layers. Combine the petroleum benzin extracts and wash with three 50-mL portions of water. Evaporate the petroleum benzin on a water bath, and dry the residue at 105° C for 30 minutes. The mass of the dried residue is not more than 4.0% of the mass of the So-dium Lauryl Sulfate.

Water $\langle 2.48 \rangle$ Not more than 5.0% (0.5 g, volumetric titration, direct titration).

Total alcohol content Dissolve about 5 g of Sodium Lauryl Sulfate, accurately weighed, in 150 mL of water and 50 mL of hydrochloric acid, and boil under a reflux condenser for 4 hours. Cool, extract with two 75-mL portions of diethyl ether, and evaporate the combined diethyl ether extracts on a water bath. Dry the residue at 105° C for 30 minutes. The mass of the residue is not less than 59.0%.

Containers and storage Containers—Well-closed containers.

Sodium Pertechnetate (^{99m}Tc) Injection

過テクネチウム酸ナトリウム (⁹⁹Tc) 注射液

Sodium Pertechnetate (99m Tc) Injection is an aqueous solution for injection. It contains technetium-99m (99m Tc) in the form of sodium pertechnetate.

It conforms to the requirements of Sodium Pertechnetate (^{99m}Tc) Injection in the Minimum Requirements for Radiopharmaceuticals.

Test for Extractable Volume of Parenteral Preparations and Insoluble Particulate Matter Test for Injections are not applied to this injection.

Description Sodium Pertechnetate (^{99m}Tc) Injection is a clear, colorless liquid.

Dibasic Sodium Phosphate Hydrate

リン酸水素ナトリウム水和物

Na₂HPO₄.12H₂O: 358.14

Dibasic Sodium Phosphate Hydrate contains not less than 98.0% of disodium hydrogen phosphate (Na₂HPO₄: 141.96), calculated on the dried basis.

Description Dibasic Sodium Phosphate Hydrate occurs as colorless or white crystals. It is odorless.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It effloresces in warm, dry air.

Identification (1) A solution of Dibasic Sodium Phosphate Hydrate (1 in 10) responds to the Qualitative Tests $\langle 1.09 \rangle$ (1) and (2) for sodium salt.

(2) A solution of Dibasic Sodium Phosphate Hydrate

(1 in 10) responds to the Qualitative Tests <1.09> (1) and (3) for phosphate.

(3) Dissolve 0.1 g of Dibasic Sodium Phosphate Hydrate in 5 mL of dilute nitric acid, warm at 70°C for 1 to 2 minutes, and add 2 mL of hexaammonium heptamolybdate TS: a yellow precipitate is formed.

pH <2.54> Dissolve 1.0 g of Dibasic Sodium Phosphate Hydrate in 50 mL of water: the pH of this solution is between 9.0 and 9.4.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Dibasic Sodium Phosphate Hydrate in 20 mL of water: the solution is clear and colorless.

(2) Chloride $\langle 1.03 \rangle$ —Dissolve 1.0 g of Dibasic Sodium Phosphate Hydrate in 7 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 0.014%).

(3) Sulfate $\langle 1.14 \rangle$ —Dissolve 0.5 g of Dibasic Sodium Phosphate Hydrate in 2 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 0.038%).

(4) Carbonate—To 2.0 g of Dibasic Sodium Phosphate Hydrate add 5 mL of water, boil, and add 2 mL of hydrochloric acid after cooling: the solution does not effervesce.

(5) Heavy metals $\langle 1.07 \rangle$ —Dissolve 2.0 g of Dibasic Sodium Phosphate Hydrate in 4 mL of acetic acid (31) and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution by adding 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(6) Arsenic $\langle 1.11 \rangle$ —Prepare the test solution with 1.0 g of Dibasic Sodium Phosphate Hydrate according to Method 1, and perform the test (not more than 2 ppm).

Loss on drying $\langle 2.41 \rangle$ 57.0 – 61.0% (1 g, at 40°C for 3 hours and then at 105°C for 5 hours, not exceeding 2 mm in sample layer).

Assay Weigh accurately about 6 g of Dibasic Sodium Phosphate Hydrate, dissolve in 50 mL of water, and then titrate $\langle 2.50 \rangle$ with 0.5 mol/L sulfuric acid VS at 15 °C until the green color of the solution changes to dark-greenish red-purple (indicator: 3 to 4 drops of methyl orange-xylenecyanol FF TS).

Each mL of 0.5 mol/L sulfuric acid VS = $142.0 \text{ mg of } Na_2HPO_4$

Containers and storage Containers—Tight containers.

Sodium Picosulfate Hydrate

ピコスルファートナトリウム水和物



C₁₈H₁₃NNa₂O₈S₂.H₂O: 499.42 Disodium 4,4'-(pyridin-2-ylmethylene)bis(phenyl sulfate) monohydrate [*10040-45-6*, anhydride]

Sodium Picosulfate Hydrate contains not less than 98.5% of sodium picosulfate ($C_{18}H_{13}NNa_2O_8S_2$: 481.41), calculated on the anhydrous basis.

Description Sodium Picosulfate Hydrate occurs as a white, crystalline powder. It is odorless and tasteless.

It is very soluble in water, soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in diethyl ether.

It is gradually colored by light.

The pH of a solution of Sodium Picosulfate Hydrate (1 in 20) is between 7.4 and 9.4.

Identification (1) Mix 5 mg of Sodium Picosulfate Hydrate with 0.01 g of 1-chloro-2,4-dinitrobenzene, and melt by gentle heating for 5 to 6 seconds. After cooling, add 4 mL of potassium hydroxide-ethanol TS: an orange-red color develops.

(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 formed.

(3) Determine the absorption spectrum of a solution of Sodium Picosulfate Hydrate (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Sodium Picosulfate Hydrate, previously dried at 105° C in vacuum for 4 hours, as directed in the potassium bromide disk method under the Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: 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 $\langle 1.09 \rangle$ for sodium salt.

Absorbance $\langle 2.24 \rangle = E_{1 \text{ cm}}^{1\%}$ (263 nm): 120 – 130 (calculated on the anhydrous basis, 4 mg, 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 clear and colorless to pale yellow.

(2) Chloride $\langle 1.03 \rangle$ —Perform the test with 0.5 g of Sodium Picosulfate Hydrate. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.028%).

(3) Sulfate $\langle 1.14 \rangle$ —Perform the test with 0.40 g of Sodium Picosulfate Hydrate. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.042%).

(4) Heavy metals <1.07>—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 $\langle 1.11 \rangle$ —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 sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 500 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-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 (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water $\langle 2.48 \rangle$ 3.0 – 4.5% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.4 g of Sodium Picosulfate Hydrate, dissolve in 50 mL of methanol, add 7 mL of acetic acid (100), and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = $48.14 \text{ mg of } C_{18}H_{13}NNa_2O_8S_2$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Sodium Polystyrene Sulfonate

ポリスチレンスルホン酸ナトリウム

Sodium Polystyrene Sulfonate is a cation exchange resin prepared as the sodium form of the sulfonated styrene divinylbenzene copolymer.

It 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 not less than 0.110 g and not more than 0.135 g of potassium (K: 39.10).

Description Sodium Polystyrene Sulfonate occurs as a yellow-brown powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95), in acetone and in diethyl ether.

Identification (1) Determine the infrared absorption spectrum of Sodium Polystyrene Sulfonate as directed in the potassium bromide disk method under the Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities

of absorption at the same wave numbers.

(2) To 1 g of Sodium Polystyrene Sulfonate add 10 mL of dilute hydrochloric acid, stir, and filter. Add ammonia TS to the filtrate to neutralize: the solution responds to the Qualitative Tests $\langle 1.09 \rangle$ for sodium salt.

Purity (1) Ammonium—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 <1.07>—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 $\langle 1.11 \rangle$ —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 supernatant liquid as the sample solution. Separately, dissolve 10 mg of styrene in acetone to make exactly 100 mL. Pipet 1 mL of this solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and determine peak areas, $A_{\rm T}$ and $A_{\rm S}$, of styrene in each solution: $A_{\rm T}$ is not larger than $A_{\rm S}$.

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-length: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

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

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 8 minutes.

System suitability—

System performance: Dissolve 20 mg each of styrene and butyl parahydroxybenzoate in 100 mL of acetone. To 5 mL of this solution add acetone to make 100 mL. When the procedure is run with 20 μ L 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.

System repeatability: When the test is repeated 6 times with $20 \,\mu\text{L}$ 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 $\langle 2.48 \rangle$ Not more than 10.0% (0.2 g, volumetric titration, direct titration).

Assay (1) Sodium—Weigh accurately about 1 g of Sodium Polystyrene Sulfonate, calculated on the anhydrous basis, in a glass-stoppered flask, add exactly 50 mL of 3 mol/L hydrochloric acid TS, shake for 60 minutes, and filter. Discard the first 20 mL of the filtarte, pipet the subsequent 5 mL of the filtrate, and add water to make exactly 100 mL. Pipet 20 mL of this solution, add water to make exactly 1000 mL, and use this solution as the sample solution. Separately, pipet a suitable quantity of Standard Sodium Stock Solution, dilute exactly with water so that each ml of the solution contains 1 to 3 μ g of sodium (Na: 22.99), and use these solutions as the standard solutions. Perform the test with the sample solution and the standard solutions as directed under Atomic Absorption Spectrophotometry $\langle 2.23 \rangle$ according to the following conditions, and calculate the amount of sodium in the sample solution using the calibration curve obtained from the standard solutions.

Gas: Combustible gas—Acetylene. Supporting gas—Air.

Lamp: A sodium hollow-cathode lamp.

Wavelength: 589.0 nm.

(2) Potassium exchange capacity—Weigh accurately about 1.5 g of Sodium Polystyrene Sulfonate, calculated on the anhydrous basis, in a glass-stoppered flask, add exactly 100 mL of Standard Potassium Stock Solution, shake for 15 minutes, and filter. Discard the first 20 mL of the filtrate, pipet the subsequent 10 mL of the filtrate, and add water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 1000 mL, and use this solution as the sample solution. Separately, pipet a suitable quantity of Standard Potassium Stock Solution, dilute with water so that each mL of the solution contains an exact amount of 1 to 5 μ g of potassium (K: 39.10), and use these solutions as the standard solutions. Perform the test with these solutions as directed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions, and calculate the amount Y (mg) of potassium in 1000 mL of the sample solution using the calibration curve obtained from the standard solution. The quantity of potassium absorbed on each g of Sodium Polystyrene Sulfonate, calculated on the anhydrous basis, is calculated from the following equation: it is between 0.110 g and 0.135 g.

Quantity (mg) of potassium (K) absorbed on 1 g of Sodium Polystyrene Sulfonate, calculated on the anhydrous basis = (X - 100Y)/M

- X: Amount (mg) of potassium in 100 mL of the Standard Potassium Stock Solution before exchange
- *M*: Mass (g) of Sodium Polystyrene Sulfonate taken, calculated on the anhydrous basis

Gas: Combustible gas—Acetylene. Supporting gas—Air. Lamp: A potassium hollow-cathode lamp. Wavelength: 766.5 nm.

Containers and storage Containers—Tight containers.

Sodium Prasterone Sulfate Hydrate

プラステロン硫酸エステルナトリウム水和物



C₁₉H₂₇NaO₅S.2H₂O: 426.50

Monosodium 17-oxoandrost-5-en- 3β -yl sulfate dihydrate [*1099-87-2*, anhydride]

Sodium Prasterone Sulfate Hydrate contains not less than 98.0% of sodium prasterone sulfate ($C_{19}H_{27}NaO_5S$: 390.47), calculated on the dried basis.

Description Sodium Prasterone Sulfate Hydrate occurs as white crystals or crystalline powder. It is odorless.

It is soluble in methanol, sparingly soluble in water and in ethanol (95), and practically insoluble in acetone and in diethyl ether.

The pH of a solution of Sodium Prasterone Sulfate Hydrate (1 in 200) is between 4.5 and 6.5.

Melting point: about 160° C (with decomposition, after drying).

Identification (1) Dissolve 0.01 g of Sodium Prasterone Sulfate Hydrate in 4 mL of ethanol (95), add 2 mL of 1,3-dinitrobenzene TS and 2 mL of a solution of sodium hydroxide (1 in 8): a red-purple color develops, and gradually changes to brown.

(2) To 10 mL of a solution of Sodium Prasterone Sulfate Hydrate (1 in 200) add 0.5 mL of bromine TS: the color of bromine TS immediately disappears.

(3) Determine the infrared absorption spectrum of Sodium Prasterone Sulfate Hydrate as directed in the potassium bromide disk method under the Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Sodium Prasterone Sulfate Hydrate (1 in 200) responds to the Qualitative Tests <1.09> for sodium salt.

Optical rotation $\langle 2.49 \rangle$ [α]_D²⁰: +10.7 - +12.1° (0.73 g, calculated on the dried basis, methanol, 20 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 0.25 g of Sodium Prasterone Sulfate Hydrate in 50 mL of water: the solution is clear and colorless.

(2) Chloride $\langle 1.03 \rangle$ —Dissolve 1.0 g of Sodium Prasterone Sulfate Hydrate in 20 mL of acetone and 20 mL of water, 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 as follows: to 0.30 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 0.011%).

(3) Sulfate $\langle 1.14 \rangle$ —To 1.2 g of Sodium Prasterone Sulfate Hydrate add 20 mL of water, shake vigorously for 5 minutes, and filter. To 10 mL of the filtrate add 20 mL of acetone, 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 20 mL of acetone, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.032%).

(4) Heavy metals $\langle 1.07 \rangle$ —Proceed with 2.0 g of Sodium Prasterone 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).

(5) Related substances—Dissolve 0.10 g of Sodium Prasterone Sulfate Hydrate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample 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 $\langle 2.03 \rangle$. Spot 5 μ L each of the sample solution and 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 water (75:22:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly a mixture of sulfuric acid and ethanol (95) (1:1) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying $\langle 2.41 \rangle$ 8.0 – 9.0% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Assay Weigh accurately about 0.25 g of Sodium Prasterone Sulfate Hydrate, dissolve in 30 mL of water. Apply this solution to a chromatographic column 10 mm in inside diameter, previously prepared by pouring 5 mL of strongly acidic ion-exchange resin (H type) for column chromatography, and elute at the rate of 4 mL per minute. Wash the chromatographic column with 100 mL of water, combine the washings with above effluent solution, and titrate $\langle 2.50 \rangle$ with 0.05 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

> Each mL of 0.05 mol/L sodium hydroxide VS = $19.52 \text{ mg of } C_{19}H_{27}NaO_5S$

Containers and storage Containers-Tight containers.

Sodium Pyrosulfite

Sodium Metabisulfite

ピロ亜硫酸ナトリウム

Na₂S₂O₅: 190.11

Sodium Pyrosulfite contains not less than 95.0% of $Na_2S_2O_5$.

Description Sodium Pyrosulfite occurs as white crystals or crystalline powder. It has the odor of sulfur dioxide.

It is freely soluble in water, very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

A solution of Sodium Pyrosulfite (1 in 20) is acid.

It is hygroscopic.

It decomposes slowly on exposure to air.

Identification A solution of Sodium Pyrosulfite (1 in 20) responds to the Qualitative Tests <1.09> 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 $\langle 1.07 \rangle$ —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 $\langle 1.10 \rangle$ —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 $\langle 1.11 \rangle$ —Dissolve 0.5 g of Sodium Pyrosulfite in 10 mL of water, heat with 1 mL of sulfuric acid on 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, and titrate $\langle 2.50 \rangle$ the excess of iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L iodine VS = $4.753 \text{ mg of } \text{Na}_2\text{S}_2\text{O}_5$

Containers and storage Containers—Tight containers. Storage—Light-resistant, preferably well-filled, and not exceeding 30°C.

Sodium Risedronate Hydrate

リセドロン酸ナトリウム水和物



 $C_7H_{10}NNaO_7P_2.2\frac{1}{2}H_2O: 350.13$ Monosodium trihydrogen 1-hydroxy-2-(pyridin-3-yl)ethane-1,1diyldiphosphonate hemipentahydrate [329003-65-8]

Sodium Risedronate Hydrate contains not less than 98.0% and not more than 102.0% of sodium risedronate ($C_7H_{10}NNaO_7P_2$: 305.09), calculated on the anhydrous basis.

Description Sodium Risedronate Hydrate occurs as a white crystalline powder.

It is soluble in water, and practically insoluble in ethanol (99.5).

It dissolves in diluted dilute sodium hydroxide TS (1 in 20).

Identification (1) Determine the absorption spectrum of a solution of Sodium Risedronate Hydrate in diluted dilute sodium hydroxide TS (1 in 20) (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Sodium Risedronate Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Sodium Risedronate Hydrate responds to the Qualitative Tests $\langle 1.09 \rangle$ (1) for sodium salt.

Purity (1) Heavy metals—To 0.50 g of Sodium Risedronate Hydrate in a quartz crucible add 0.50 g of magnesium oxide, mix, heat until the content becomes a light gray while mixing occasionally with a glass rod, then incinerate at 800°C. After cooling, dissolve the residue with 3 mL of hydrochloric acid, and add 3 mL of water. Adjust this solution to pH 8.5 with ammonia TS, then adjust to pH 4 with acetic acid (100), and adjust the pH to 3.4 with dilute hydrochloric acid. Filter the solution into a Nessler tube using a filter paper, rinse the crucible and filter with water, add the rinsings to the Nessler tube, then add water to make 50 mL, and use this solution as the test solution. Separately, to 1.0 mL of Standard Lead Solution add 0.50 g of magnesium oxide, dryness at 110°C, and proceed with the residue in the same manner as for the test solution, and use the solution so obtained as the control solution. To the test and control solutions add 1 drop each of sodium sulfate TS, mix, and allow to stand for 5 minutes, and compare the colors of both 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).

(2) Arsenic $\langle 1.11 \rangle$ —Prepare the test solution by dissolving 1.0 g of Sodium Risedronate Hydrate in 5 mL of a solution of sodium hydroxide (1 in 5), and perform the test (not more than 2 ppm).

(3) Related substance 1—Dissolve 50 mg of Sodium Risedronate Hydrate in 1.5 mL of 0.2 mol/L sodium hydroxide TS, add the mobile phase to make 25 mL, and use this solution as the sample solution. Pipet 2.5 mL of the sample solution, and add the mobile phase 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 exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than risedronic acid obtained from the sample solution is not larger than the peak area of risedronic 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.

Time span of measurement: About 2 times as long as the retention time of risedronic acid, beginning after the solvent peak.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of risedronic acid are not less than 4500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risedronic acid is not more than 5.0%.

(4) Related substance 2—Dissolve 0.10 g of Sodium Risedronate Hydrate in 3 mL of 0.2 mL/L sodium hydroxide TS, add the diluting solution below to make 50 mL, and use this solution as the sample solution. Pipet 2.5 mL of the sample solution, and add the diluting solution to make exactly 50 mL. Pipet 2 mL of this solution, add the diluting solution to make exactly 50 mL. Perform the test with exactly 50 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than risedronic acid obtained from the sample solution is not larger than the peak area of risedronic acid from the standard solution.

Diluting solution: Dissolve 0.11 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate and 2.47 g of tetradecyl trimethylammonium bromide in 1000 mL of water, and adjust to pH 6.5 with 0.2 mL/L sodium hydroxide TS. To 700 mL of this solution add 300 mL of acetonitrile. *Operating conditions—*

Detector: An ultraviolet absorption photometer (wave-

length: 263 nm). Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about $25^{\circ}C$.

Mobile phase: Dissolve 0.14 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate, 3.16 g of tetradecyl trimethylammonium bromide, 4.81 g of ammonium dihydrogen phosphate and 2.93 g of diammonium hydrogen phosphate in 1280 mL of water, and add 720 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of risedronic acid is about 5 minutes.

Time span of measurement: About 10 times as long as the retention time of risedronic acid, beginning after the solvent peak.

System suitability—

System performance: When the procedure is run with 50 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of risedronic acid are not less than 5000 and not more than 1.4, respectively.

System repeatability: When the test is repeated 6 times with 50 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak

area of risedronic acid is not more than 2.0%.

(5) Residual solvent Being specified separately.

Water $\langle 2.48 \rangle$ 11.9 – 13.9% (40 mg, volumetric titration, direct titration. Use a mixture of formamide for Karl Fisher method and methanol for Karl Fisher method (1:1) instead of methanol for Karl Fisher method).

Assay Weigh accurately about 50 mg of Sodium Risedronate Hydrate, dissolve in 1.5 mL of 0.2 mL/L sodium hydroxide TS, and add the mobile phase to make exactly 25 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, then add the mobile phase to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Risedronic Acid RS (separately determine the water <2.48> using 80 mg, in the same manner as Sodium Risedronate Hydrate), dissolve in 3 mL of 0.2 mol/L sodium hydroxide TS, and add the mobile phase to make exactly 25 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, then add the mobile phase to make 25 mL, and use this solution as the standard solution. Perform the test with $20 \,\mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of risedronic acid to that of the internal standard.

- Amount (mg) of sodium risedronate $(C_7H_{10}NNaO_7P_2)$ = $M_S \times Q_T/Q_S \times 1.078$
- $M_{\rm S}$: Amount (mg) of Risedronic Acid RS, calculated on the anhydrous basis

Internal standard solution—A solution of sodium benzoate in the mobile phase (1 in 125).

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-length: 263 nm).

Column: A polyether ether ketone column 4 mm in inside diameter and 25 cm in length, packed with quaternary alkylaminated styrene-divinylbenzene copolymer for liquid chromatography (10 μ m in particle diameter).

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

Mobile phase: Dissolve 1.8 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 1000 mL of water, and adjust to pH 9.5 with 0.2 mL/L sodium hydroxide TS.

Flow rate: Adjust the flow rate so that the retention time of risedronic acid is about 14 minutes.

System suitability-

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the internal standard and risedronic acid 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 $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risedronic acid is not more than 1.0%.

Containers and storage Containers-Well-closed containers.

Sodium Risedronate Tablets

リセドロン酸ナトリウム錠

Sodium Risedronate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of sodium risedronate ($C_7H_{10}NNaO_7P_2$: 305.09).

Method of preparation Prepare as directed under Tablets, with Sodium Risedronate Hydrate.

Identification Powder Sodium Risedronate Tablets. To a portion of the powder, equivalent to 2.5 mg of sodium risedronate ($C_7H_{10}NNaO_7P_2$) according to the labeled amount, add 50 mL of diluted dilute sodium hydroxide TS (1 in 20), shake, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.2 μ m. Discard the first 2 mL of the filtrate, and determine the absorption spectrum of the subsequent filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 260 nm and 264 nm.

Uniformity of dosage units $\langle 6.02 \rangle$ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Sodium Risedronate Tablets add exactly 10 mL of the mobile phase, shake, and allow to stand for 10 minutes. Disperse the particles for 10 minutes with the aid of ultrasonic waves with occasional shaking, then centrifuge, and filter the supenatant liquid through a membrane filter with a pore size not exceeding $0.2 \,\mu\text{m}$. Discard the first 1 mL of the filtrate, pipet exactly VmL of the subsequent filtrate, equivalent to about 1.75 mg of sodium risedronate $(C_7H_{10}NNaO_7P_2)$, add exactly 1 mL of the internal standard solution and the mobile phase to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 70 mg of Risedronic Acid RS (separately determine the water <2.48> using 80 mg, in the same manner as Sodium Risedronate Hydrate), dissolve in 3 mL of 0.2 mol/L sodium hydroxide TS, and add the mobile phase to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 2 mL of the internal standard solution, then add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with $20 \,\mu L$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of risedronic acid to that of the internal standard.

Amount (mg) of sodium risedronate (
$$C_7H_{10}NNaO_7P_2$$
)
= $M_5 \times Q_T/Q_5 \times 1/V \times 1/4 \times 1.078$

 $M_{\rm S}$: Amount (mg) of Risedronic Acid RS, calculated on the anhydrous basis

Internal standard solution—A solution of sodium benzoate in the mobile phase (7 in 2000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Sodium Risedronate Hydrate. System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating con-

ditions, the internal standard and risedronic acid 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 $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of risedronic acid to that of the internal standard is not more than 1.0%.

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 20 minutes of Sodium Risedronate Tablets is not less than 80%.

Start the test with 1 tablet of Sodium Risedronate Tablets, withdraw 10 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding $0.45 \,\mu\text{m}$. Discard the first 2 mL of the filtrate, pipet $V \,\mathrm{mL}$ of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 2.8 μg of sodium risedronate (C₇H₁₀NNaO₇P₂) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Risedronic Acid RS (separately determine the water $\langle 2.48 \rangle$ using 80 mg in the same manner as Sodium Risedronate Hydrate), dissolve in 3 mL of 0.2 mol/L sodium hydroxide TS, and add water to make exactly 50 mL. Pipet 5 mL of this 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 exactly $200 \,\mu L$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of risedronic acid of both solutions.

Dissolution rate (%) with respect to the labeled amount of sodium risedronate ($C_7H_{10}NNaO_7P_2$)

- $= M_{\rm S} \times A_{\rm T}/A_{\rm S} \times V'/V \times 1/C \times 9/2 \times 1.078$
- $M_{\rm S}$: Amount (mg) of Risedronic Acid RS, calculated on the anhydrous basis
- C: Labeled amount (mg) of sodium risedronate $(C_7H_{10}NNaO_7P_2)$ in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 263 nm).

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

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

Mobile phase: Dissolve 0.15 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate, 3.36 g of tetradecyl trimethylammonium bromide, 5.11 g of ammonium dihydrogen phosphate and 3.11 g of diammonium hydrogen phosphate in 1360 mL of water, and add 640 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of risedronic acid is about 12 minutes.

System suitability—

System performance: When the procedure is run with 200 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of risedronic acid are not less than 5000

and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with $200 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of risedronic acid is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Sodium Risedronate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of sodium risedronate (C₇H₁₀NNaO₇P₂), add exactly 10 mL of the internal standard solution, add 190 mL of the mobile phase, shake, and allow to stand for 10 minutes. Disperse the particles with the aid of ultrasonic waves with occasional shaking, then centrifuge, and filter through a membrane filter with a pore size not exceeding $0.2 \,\mu\text{m}$. Discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of Risedronic Acid RS (separately determine the water <2.48> using 80 mg, in the same manner as Sodium Risedronate Hydrate), dissolve in 3 mL of 0.2 mol/L sodium hydroxide TS, add exactly 10 mL of the internal standard solution, then add the mobile phase to make 200 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Sodium Risedronate Hydrate.

Amount (mg) of sodium risedronate (
$$C_7H_{10}NNaO_7P_2$$
)
= $M_5 \times Q_T/Q_5 \times 1.078$

 $M_{\rm S}$: Amount (mg) of Risedronic Acid RS, calculated on the anhydrous basis

Internal standard solution—A solution of sodium benzoate in the mobile phase (1 in 100).

Containers and storage Containers—Well-closed containers.

Sodium Salicylate

サリチル酸ナトリウム



C₇H₅NaO₃: 160.10 Monosodium 2-hydroxybenzoate [54-21-7]

Sodium Salicylate, when dried, contains not less than 99.5% of $C_7H_5NaO_3$.

Description Sodium Salicylate occurs as white, crystals or crystalline powder.

It is very soluble in water, freely soluble in acetic acid (100), and soluble in ethanol (95).

It is gradually colored by light.

Identification (1) Determine the infrared absorption spectrum of Sodium Salicylate, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Sodium Salicylate (1 in 20) responds to the Qualitative Tests <1.09> for sodium salt.

pH $\langle 2.54 \rangle$ The pH of a solution of 2.0 g of Sodium Salicylate in 20 mL of water is between 6.0 and 8.0.

Purity (1) Clarity of solution—Dissolve 1.0 g of Sodium Salicylate in 10 mL of water: the solution is clear, and its absorbance at 420 nm determined as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$ is not more than 0.02.

(2) Chloride $\langle 1.03 \rangle$ —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 using this solution as the test solution. 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 <1.07>—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 $\langle 1.11 \rangle$ —To 1.0 g of Sodium Salicylate in a decomposition flask add 5 mL of nitric acid and 2 mL of sulfuric acid, and heat carefully 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 until the solution is colorless to pale yellow. Repeat the procedure of adding nitric acid and hydrogen peroxide (30) and heating, if necessary. After cooling, add 2 mL of a saturated solution of ammonium oxalate monohydrate, and heat until white fumes are evolved. After cooling, add water to make 5 mL, and perform the test with this solution (not more than 2 ppm).

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 2 hours).

Assay Weigh accurately about 0.3 g of Sodium Salicylate, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 16.01 mg of C₇H₅NaO₃

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Sodium Starch Glycolate

デンプングリコール酸ナトリウム

[9063-38-1]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (\bullet).

Sodium Starch Glycolate is the sodium salt of a car-

boxymethyl ether of starch or of a cross-linked carboxymethyl ether of starch.

There are two neutralization types of Sodium Starch Glycolate, Type A and Type B, and their insoluble matter in a mixture of ethanol (99.5) and water (8:2), when dried, contains not less than 2.8% and not more than 4.2%, and not less than 2.0% and not more than 3.4% of sodium (Na: 22.99), respectively.

◆The label states the type of neutralization.

•**Description** Sodium Starch Glycolate occurs as a white powder, and has a characteristic salty taste.

It practically insoluble in ethanol (99.5).

It swells with water, and becomes viscous, pasty liquid. It is hygroscopic. \bullet

Identification (1) Acidify 5 mL of a solution of Sodium Starch Glycolate (1 in 500) with dilute hydrochloric acid, then add one drop of iodine TS, and stir: a blue to violet color is produced.

•(2) Determine the infrared absorption spectrum of Sodium Starch Glycolate as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) The sample solution obtained in the Purity (2) responds to the Qualitative Tests $\langle 1.09 \rangle$ (2) for sodium salt. Perform the test using 2 mL of the sample solution and 4 mL of potassium hexahydroxoantimonate (V) TS.

pH $\langle 2.54 \rangle$ To 1 g of Sodium Starch Glycolate add 30 mL of water and stir: the pH of the resulting suspension of Type A is 5.5 – 7.5, and that of Type B is 3.0 – 5.0.

Purity (1) Heavy metals (1.07)—Proceed with 1.0 g of Sodium Starch Glycolate 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) Iron

(i) Sample solution Take 2.5 g of Sodium Starch Glycolate in a silica or platinum crucible, add 2 mL of 5 mol/L sulfuric acid TS. Heat on a water bath, then ignite cautiously with a gas burner or preferably in an electric furnace at 600 ± 25 °C, and incinerate the residue completely. Allow to cool, add a few drops of 1 mol/L sulfuric acid TS, and heat and ignite as above. Allow to cool, add a few drops of a mmonium carbonate TS, evaporate to dryness on a water bath, and heat and ignite as above. After cooling, dissolve the residue by adding 50 mL of water.

(ii) Standard solution Weigh accurately 863.4 mg of ammonium iron (III) sulfate dodecahydrate, dissolve in water, add 25 mL of 1 mol/L sulfuric acid TS, and add water to make exactly 500 mL. Pipet 10 mL of this solution, and add water to make exactly 100 mL. Pipet 5 mL of this solution, and add water to make exactly 100 mL. Each mL of this solution contains $1.0 \mu g$ of iron (Fe).

(iii) Procedure Pipet 10 mL each of the sample solution and standard solution, and to each solution add 2 mL of citric acid solution (1 in 5) and 0.1 mL of thioglycolic acid. Then add ammonia solution (28) dropwise to render the solution alkaline, using litmus paper as an indicator. Add water to make 20 mL, and use these solutions as the test solution and the control solution, respectively. Allow these solutions to stand for 5 minutes, and compare the color of the solutions using white background: the color of the test solution is not deeper than that of the control solution (not more than 20 ppm).

(3) Sodium glycolate—Conduct this procedure without exposure to light, using light-resistant vessels.

(i) Sample solution Weigh accurately 0.200 g of Sodium Starch Glycolate in a beaker, add 4 mL of 6 mol/L acetic acid TS and 5 mL of water, and stir to dissolve. Add 50 mL of acetone and 1 g of sodium chloride, stir, and filter through a filter paper previously soaked with acetone. Rinse the beaker and the filter paper with acetone, combine the filtrate and washings, and add acetone to make exactly 100 mL. Allow to stand for 24 hours, and use the supernatant liquid as the sample solution.

(ii) Standard solution To exactly 0.310 g of glycolic acid, previously dried in a desiccator (silica gel) for 18 hours, add water to dissolve to make exactly 500 mL. Pipet 5 mL of this solution, add 4 mL of 6 mol/L acetic acid TS, and allow to stand for 30 minutes. Add 50 mL of acetone and 1 g of so-dium chloride, proceed as (i) above, and use the supernatant liquid as the standard solution.

(iii) Procedure Pipet 2.0 mL each of the sample solution and standard solution into 25-mL stoppered test tubes, and heat on a water bath for 20 minutes to remove acetone. After cooling, add 20.0 mL of 2,7-dihydroxynaphthalene TS to the residue, stopper the test tube, and heat on a water bath for 20 minutes. Cool under running water, and transfer whole quantity of the content to a 25-mL volumetric flask. Maintain the flask under running water, and add sulfuric acid to make 25 mL. Within 10 minutes, determine the absorbance of these solutions at 540 nm using water as the blank as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$; the absorbance of the standard solution (not more than 2.0%).

(4) Sodium chloride—Weigh accurately about 0.5 g of Sodium Starch Glycolate in a beaker, disperse in 100 mL of water, and add 1 ml of nitric acid. Titrate $\langle 2.50 \rangle$ with 0.1 mol/L silver nitrate VS (potentiometric titration): the amount of sodium chloride (NaCl: 58.44) is not more than 7.0%.

Each mL of 0.1 mol/L silver nitrate VS = 5.844 mg of NaCl

Loss on drying $\langle 2.41 \rangle$ Not more than 10.0% (1 g, 130°C, 90 minutes).

Assay To about 1 g of Sodium Starch Glycolate add 20 mL of a mixture of ethanol (99.5) and water (8:2), stir for 10 minutes, and filter. Repeat this procedure until no more turbidity is produced by adding silver nitrate TS, and dry the residue on the filter paper at $105 \,^{\circ}$ C to constant mass. Weigh accurately 0.7 g of the mass, add 80 mL of acetic acid (100), and heat the mixture under a reflux condenser on a water bath for 2 hours. After cooling, titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration).

Content (%) of sodium (Na) = $(V \times 2.299 \times 100)/M$

- V: Consumed amount (mL) of 0.1 mol/L perchloric acid VS
- M: Mass (mg) of the dried residue

◆Containers and storage Containers—Tight containers. ◆

Dried Sodium Sulfite

乾燥亜硫酸ナトリウム

Na₂SO₃: 126.04

Dried Sodium Sulfite contains not less than 97.0% of Na_2SO_3 .

Description Dried Sodium Sulfite is white crystals or powder. It is odorless.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

The pH of a solution of Dried Sodium Sulfite (1 in 10) is about 10.

It gradually changes in moist air.

Identification An aqueous solution of Dried Sodium Sulfite (1 in 20) responds to the Qualitative Tests <1.09> for sodium salt and sulfite.

Purity (1) Thiosulfate—Dissolve 1.0 g of Dried Sodium Sulfite in 15 mL of water, add gradually 5 mL of hydrochloric acid, shake, and allow to stand for 5 minutes: no turbidity is produced.

(2) Heavy metals $\langle 1.07 \rangle$ —Dissolve 1.0 g of Dried Sodium Sulfite in 5 mL of water, add 2 mL of hydrochloric acid gradually, and evaporate the mixture on a water bath to dryness. Add 3 mL of boiling water and 1 mL of hydrochloric acid to the residue, and again evaporate to dryness on a water bath. Dissolve the residue in 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 as follows: evaporate 3 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 20 ppm).

(3) Arsenic $\langle 1.11 \rangle$ —Dissolve 0.5 g of Dried Sodium Sulfite in 5 mL of water, add 1 mL of sulfuric acid, and evaporate on a sand bath until white fumes are evolved. Add water to make 5 mL, take this solution as the sample solution, and perform the test (not more than 4 ppm).

Assay Weigh accurately about 0.2 g of Dried Sodium Sulfite, transfer immediately to an iodine flask containing exactly 50 mL of 0.05 mol/L iodine VS, stopper, shake, and allow to stand for 5 minutes in a dark place. Add 1 mL of hydrochloric acid, and titrate $\langle 2.50 \rangle$ the excess iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L iodine VS = $6.302 \text{ mg of } Na_2SO_3$

Containers and storage Containers-Tight containers.

Sodium Thiosulfate Hydrate

チオ硫酸ナトリウム水和物

 $Na_2S_2O_3.5H_2O: 248.18$

Sodium Thiosulfate Hydrate, when dried, contains not less than 99.0% and not more than 101.0% of so-dium thiosulfate (Na₂S₂O₃: 158.11).

Description Sodium Thiosulfate Hydrate occurs as color-less, crystals or crystalline powder. It is odorless.

It is very soluble in water, and practically insoluble in ethanol (99.5).

It effloresces in dry air, and is deliquescent in moist air.

Identification (1) A solution of Sodium Thiosulfate Hydrate (1 in 10) responds to the Qualitative Tests $\langle 1.09 \rangle$ for thiosulfate.

(2) A solution of Sodium Thiosulfate Hydrate (1 in 10) responds to the Qualitative Tests $\langle 1.09 \rangle$ for sodium salt.

pH $\langle 2.54 \rangle$ Dissolve 1.0 g of Sodium Thiosulfate Hydrate 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 $\langle 1.07 \rangle$ —Dissolve 1.0 g of Sodium Thiosulfate Hydrate in 10 mL of water, add slowly 5 mL of dilute hydrochloric acid, and evaporate on 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. Cool, add 1 drop of phenolphthalein TS, and add dropwise 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 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).

(3) Calcium—Dissolve 1.0 g of Sodium Thiosulfate 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 $\langle 1.11 \rangle$ —To 0.40 g of Sodium Thiosulfate add 3 mL of nitric acid and 5 mL of water, evaporate on 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 $\langle 2.41 \rangle$ 32.0 – 37.0% (1 g, in vacuum, 40 – 45°C, 16 hours).

Assay Weigh accurately about 0.4 g of Sodium Thiosulfate, previously dried, dissolve in 30 mL of water, and titrate $\langle 2.50 \rangle$ with 0.05 mol/L iodine VS (indicator: 1 mL of starch TS).

Each mL of 0.05 mol/L iodine VS = $15.81 \text{ mg of } \text{Na}_2\text{S}_2\text{O}_3$

Containers and storage Containers—Tight containers.

Sodium Thiosulfate Injection

チオ硫酸ナトリウム注射液

Sodium Thiosulfate Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of sodium thiosulfate hydrate (Na₂S₂O₃.5H₂O: 248.18).

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Method of preparation Prepare as directed under Injections, with Sodium Thiosulfate Hydrate.

Description Sodium Thiosulfate Injection is a clear, color-less liquid.

Identification Sodium Thiosulfate Injection responds to the Qualitative Tests <1.09> for sodium salt and for thiosulfate.

Bacterial endotoxins <4.01> Less than 0.01 EU/mg.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter $\langle 6.06 \rangle$ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of Sodium Thiosulfate Injection, equivalent to about 0.5 g of sodium thiosulfate hydrate (Na₂S₂O₃.5H₂O), add water to make 30 mL, and titrate $\langle 2.50 \rangle$ with 0.05 mol/L iodine VS (indicator: 1 mL of starch TS).

Each mL of 0.05 mol/L iodine VS = 24.82 mg of Na₂S₂O₃.5H₂O

Containers and storage Containers—Hermetic containers.

Sodium Valproate

バルプロ酸ナトリウム



C₈H₁₅NaO₂: 166.19 Monosodium 2-propylpentanoate [*1069-66-5*]

Sodium Valproate, when dried, contains not less than 98.5% and not more than 101.0% of $C_8H_{15}NaO_2$.

Description Sodium Valproate occurs as a white, crystalline powder.

It is very soluble in water, freely soluble in ethanol (99.5) and in acetic acid (100).

It is hygroscopic.

Identification (1) To 5 mL of a solution of Sodium Valproate (1 in 20) add 1 mL of a solution of cobalt (II) nitrate hexahydrate (1 in 20) and warm on a water bath: a purple precipitate is formed.

(2) Dissolve 0.5 g of Sodium Valproate in 5 mL of water, add 5 mL of diethyl ether and 1 mL of 2 mol/L hydrochloric acid TS, and shake vigorously for 1 minute. Separate the diethyl ether layer, dehydrate with anhydrous sodium sulfate, and filter. Evaporate the solvent of the filtrate, determine the infrared spectrum of the residue as directed in the liquid film method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH $\langle 2.54 \rangle$ 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) Heavy metals $\langle 1.07 \rangle$ —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 filtrate 25 mL with ammonia TS, 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 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).

(2) Related substances—Dissolve 0.10 g of Sodium Valproate in 10 mL of a mixture of formic acid and methyl acetate (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of formic acid and methyl acetate (1:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly $2 \mu L$ each of the sample solution and standard solution as directed under Gas Chromatography $\langle 2.02 \rangle$ according to the following conditions. Determine each peak area of both solutions by automatic integration method: the total area of the peaks other than the peak of valproic acid from the standard solution.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography (150 to 180 μ m 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 about 7 minutes.

Time span of measurement: About 2 times as long as the retention time of valproic acid, beginning after the solvent peak.

System suitability—

System performance: To 2 mL of the sample solution and $8 \,\mu\text{L}$ of *n*-valerianic acid, add a mixture of formic acid and methyl acetate (1:1) to make 10 mL. When the procedure is run with $2 \,\mu\text{L}$ of this solution under the above operating conditions, *n*-valerianic acid and valproic acid are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: Pipet 2 mL of the standard solution and add a mixture of formic acid and methyl acetate (1:1) to make exactly 10 mL. When the test is repeated 6 times with 2 μ L of this solution under the above operating conditions, the relative standard deviation of the peak area of valproic acid is not more than 5.0%.

Loss on drying $\langle 2.41 \rangle$ 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 $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potenti-

ometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = $16.62 \text{ mg of } C_8H_{15}NaO_2$

Containers and storage Containers—Tight containers.

Sodium Valproate Syrup

バルプロ酸ナトリウムシロップ

Sodium Valproate Syrup contains not less than 95.0% and not more than 105.0% of the labeled amount of sodium valproate ($C_8H_{15}NaO_2$: 166.19).

Method of preparation Prepare as directed under Syrups, with Sodium Valproate.

Identification To a volume of Sodium Valproate Syrup, equivalent to 50 mg of Sodium Valproate according to the labeled amount, add water to make 10 mL. To 5 mL of this solution add 1 mL of a solution of cobalt (II) nitrate hexahydrate (1 in 20) and warm on a water bath: a purple precipitate is formed.

Microbial limit <4.05> The acceptance criteria of TAMC and TYMC are 10² CFU/mL and 10¹ CFU/mL, respectively. *Escherichia coli* is not observed.

Assay Pipet a volume of Sodium Valproate Syrup, equivalent to about 0.1 g of sodium valproate ($C_8H_{15}NaO_2$) and add water to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 5 mL of internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of sodium valproate for assay, previously dried at 105 °C for 3 hours, dissolve in water to make exactly 50 mL. Pipet 20 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 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of valproic acid to that of the internal standard.

Amount (mg) of sodium valproate (C₈H₁₅NaO₂) = $M_{\rm S} \times Q_{\rm T}/Q_{\rm S} \times 2$

 $M_{\rm S}$: Amount (mg) of sodium valproate for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in the mobile phase (1 in 50,000). *Operating conditions*—

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

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

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

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogenphosphate TS, pH 3.0 and acetonitrile (1:1).

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

System suitability—

System performance: When the procedure is run with 10

 μ L of the standard solution under the above operating conditions, the internal standard and valproic acid are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of valproic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Sodium Valproate Tablets

バルプロ酸ナトリウム錠

Sodium Valproate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of sodium valproate ($C_8H_{15}NaO_2$: 166.19).

Method of preparation Prepare as directed under Tablets, with Sodium Valproate.

Identification To a quantity of powdered Sodium Valproate Tablets, equivalent to 0.5 g of Sodium Valproate according to the labeled amount, add 10 mL of water, shake well, and centrifuge. To 5 mL of the supernatant liquid add 1 mL of a solution of cobalt (II) nitrate hexahydrate (1 in 20) and warm on a water bath: a purple precipitate is formed.

Uniformity of dosage units $\langle 6.02 \rangle$ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Sodium Valproate Tablets add 7V/10 mL of the mobile phase, shake vigorously, add the mobile phase to make exactly V mL so that each mL contains about 1 mg of sodium valproate (C₈H₁₅NaO₂), and centrifuge. Filter the supernatant liquid, pipet 20 mL of the filtrate, add exactly 5 mL of the internal standard solution, shake vigorously, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of sodium valproate ($C_8H_{15}NaO_2$) = $M_S \times Q_T/Q_S \times V/100$

 $M_{\rm S}$: Amount (mg) of sodium valproate for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in the mobile phase (1 in 50,000).

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Sodium Valproate Tablets is not less than 85%.

Start the test with 1 tablet of Sodium Valproate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding $0.45 \,\mu$ m. 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 0.11 mg of sodium valproate (C₈H₁₅NaO₂) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 56 mg of sodium valproate for assay, previously dried at 105°C for

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3 hours, and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL of the solution, and use this solution as the standard solution. Perform the test with exactly 50 μ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.0I \rangle$ according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of the valproic acid in each solution.

Dissolution rate (%) with respect to the labeled amount of sodium valproate ($C_8H_{15}NaO_2$)

 $= M_{\rm S} \times A_{\rm T}/A_{\rm S} \times V'/V \times 1/C \times 180$

 $M_{\rm S}$: Amount (mg) of sodium valproate for assay

C: Labeled amount (mg) of sodium valproate (C₈H₁₅NaO₂) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability-

System performance: When the procedure is run with 50 μ L of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of valproic acid are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with $50 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of valproic acid is not more than 1.5%.

Assay Weigh accurately the mass of not less than 20 Sodium Valproate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.2 g of sodium valproate (C₈H₁₅NaO₂), add about 160 mL of the mobile phase, shake well, add the mobile phase to make exactly 200 mL, and centrifuge. Filter the supernatant liquid, pipet 20 mL of the filtrate, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of sodium valproate for assay, previously dried at 105°C for 3 hours, and dissolve in the mobile phase to make exactly 100 mL. Pipet 20 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 $10 \,\mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of valproic acid to that of the internal standard.

Amount (mg) of sodium valproate (C₈H₁₅NaO₂) = $M_{\rm S} \times Q_{\rm T}/Q_{\rm S} \times 2$

 $M_{\rm S}$: Amount (mg) of sodium valproate for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in the mobile phase (1 in 50,000). *Operating conditions*—

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

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

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

Mobile phase: A mixture of 0.05 mol/L sodium dihydro-

gen phosphate TS, pH 3.0 and acetonitrile (1:1).

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

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the internal standard and valproic acid are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of valproic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Sorbitan Sesquioleate

ソルビタンセスキオレイン酸エステル

Sorbitan Sesquioleate is a mixture of monoester and diester of sorbitol anhydride, partially esterified with oleic acid.

Description Sorbitan Sesquioleate is a pale yellow to light yellow-brown, viscous oily liquid. It has a faint, characteristic odor and a slightly bitter taste.

It is freely soluble in diethyl ether, slightly soluble in ethanol (95), and very slightly soluble in methanol.

It is dispersed as fine oily drops in water.

Identification (1) To 0.5 g of Sorbitan Sesquioleate add 5 mL of ethanol (95) and 5 mL of dilute sulfuric acid, and heat on a water bath for 30 minutes. Cool, shake with 5 mL of petroleum ether, and allow to stand, and separate the upper layer and the lower layer. Shake 2 mL of the lower layer with 2 mL of freshly prepared catechol solution (1 in 10), then with 5 mL of sulfuric acid: a red to red-brown color develops.

(2) Heat the upper layer obtained in (1) on a water bath, and evaporate petroleum ether. To the residue add 2 mL of diluted nitric acid (1 in 2), and then add 0.5 g of potassium nitrite between 30° C and 35° C with stirring: the solution develops an opalescence, and, when cooled, crystals are formed.

Specific gravity <1.13> d_{25}^{25} : 0.960 – 1.020

Saponification value <1.13> 150 – 168

Purity (1) Acidity—To 2.0 g of Sorbitan Sesquioleate add 50 mL of neutralized ethanol, and heat on a water bath nearly to boiling with stirring once or twice. Cool, add 4.3 mL of 0.1 mol/L sodium hydroxide VS and 5 drops of phenolphthalein TS: a red color develops.

(2) Heavy metals $\langle 1.07 \rangle$ —Proceed with 1.0 g of Sorbitan Sesquioleate 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 $\langle 1.11 \rangle$ —Prepare the test solution with 1.0 g of Sorbitan Sesquioleate according to Method 2, and perform the test (not more than 2 ppm).

Water <2.48> Not more than 3.0% (1 g, volumetric titra-

tion, direct titration, stir for 30 minutes).

Residue on ignition $\langle 2.44 \rangle$ Not more than 1.0% (1 g).

Containers and storage Containers—Tight containers.

D-Sorbitol

D-ソルビトール



C₆H₁₄O₆: 182.17 D-Glucitol [*50-70-4*]

D-Sorbitol, when dried, contains not less than 97.0% of $C_6H_{14}O_6$.

Description D-Sorbitol occurs as white granules, powder, or crystalline masses. It is odorless, and has a sweet taste with a cold sensation.

It is very soluble in water, sparingly soluble in ethanol (95), and practically insoluble in diethyl ether.

It 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 develops, 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 develops.

(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 separator, 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 on 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 $97^{\circ}C$ and $101^{\circ}C$.

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 $\langle 1.03 \rangle$ —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 $\langle 1.14 \rangle$ —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 $\langle 1.07 \rangle$ —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

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develops.

(6) Arsenic $\langle 1.11 \rangle$ —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 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 the flask in 20 mL of iron (III) sulfate TS, filter through the glass filter, and wash with water. Combine the filtrate and the washings, heat at 80°C, and titrate $\langle 2.50 \rangle$ with 0.02 mol/L potassium permanganate VS: not more than 6.3 mL of volume for titration consumed or consumption is required.

(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 $\langle 2.41 \rangle$ Not more than 2.0% (0.5 g, in vacuum, phosphorus (V) oxide, 80°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.02% (5 g).

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 the solution into an iodine flask, add exactly 50 mL of potassium periodate TS, and heat for 15 minutes in a water bath. Cool, 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 $\langle 2.50 \rangle$ with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium thiosulfate VS = 1.822 mg of C₆H₁₄O₆

Containers and storage Containers—Tight containers.

D-Sorbitol Solution

D-ソルビトール液

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. It is odorless, and has a sweet taste.

It is miscible with water, with ethanol (95), with glycerin and with propylene glycol.

It sometimes separates crystalline masses.

Identification (1) To a volume of D-Sorbitol Solution, equivalent to 0.7 g of D-Sorbitol according to the labeled amount, add 2 mL of iron (II) sulfate TS and 1 mL of a solution of sodium hydroxide (1 in 5): a blue-green color develops, but no turbidity is produced.

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(2) To a volume of D-Sorbitol Solution, equivalent to 1 g of D-Sorbitol according to the labeled amount, add water to make 20 mL. To 1 mL of this solution add 1 mL of a freshly prepared solution of catechol (1 in 10), mix well, then add rapidly 2 mL of sulfuric acid, and mix: a reddish purple to red-purple color immediately develops.

Purity (1) Acidity or alkalinity—D-Sorbitol Solution is neutral.

(2) Chloride $\langle 1.03 \rangle$ —Proceed with a volume of D-Sorbitol Solution, equivalent to 2.0 g of D-Sorbitol according to the labeled amount, and perform the test. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.005%).

(3) Sulfate $\langle 1.14 \rangle$ —To a volume of D-Sorbitol Solution, equivalent to 4.0 g of D-Sorbitol according to the labeled amount, and perform the test. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.006%).

(4) Heavy metals $\langle 1.07 \rangle$ —Proceed with a volume of D-Sorbitol Solution, equivalent to 5.0 g of D-Sorbitol according to the labeled amount, and according to Method 1, perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (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, 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 $\langle 1.11 \rangle$ —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 boil gently for 3 minutes. After cooling, filter the 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 alkalinity, and filter the washings through the glass filter. Dissolve the precipitate in the flask in 20 mL of iron (III) sulfate TS, filter through the glass filter, and wash the filter with water. Combine the filtrate and the washings, heat at 80°C, and titrate $\langle 2.50 \rangle$ with 0.02 mol/L potassium permanganate VS is required.

(8) Sugars—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 of a water bath, if necessary, add 8 mL of dilute hydrochloric acid, and heat 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).

Residue on ignition $\langle 2.44 \rangle$ Measure 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 mg.

Assay Measure 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, add exactly 50 mL of potassium periodate TS, and heat for 15 minutes in a water bath. Cool, 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 $\langle 2.50 \rangle$ with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination.

> Each mL of 0.1 mol/L sodium thiosulfate VS = 1.822 mg of C₆H₁₄O₆

Containers and storage Containers—Tight containers.

Soybean Oil

Oleum Sojae

ダイズ油

Soybean Oil is the fixed oil obtained from the seeds of *Glycine max* Merrill (*Leguminosae*).

Description Soybean Oil is a clear, pale yellow oil. It is odorless or has a slight odor, and has a bland taste.

It is miscible with diethyl ether and with petroleum ether. It is slightly soluble in ethanol (95), and practically insoluble in water.

It congeals between -10° C and -17° C. Congealing point of the fatty acids: $22 - 27^{\circ}$ C

Specific gravity $\langle 1.13 \rangle$ d_{25}^{25} : 0.916 - 0.922

Acid value <1.13> Not more than 0.2.

Saponification value <1.13> 188 – 195

Unsaponifiable matter <1.13> Not more than 1.0%.

Iodine value <1.13> 126 - 140

Containers and storage Containers-Tight containers.

Spectinomycin Hydrochloride Hydrate

スペクチノマイシン塩酸塩水和物



 $C_{14}H_{24}N_2O_7.2HCl.5H_2O: 495.35$ (2R,4aR,5aR,6S,7S,8R,9S,9aR,10aS)-4a,7,9-Trihydroxy-2-methyl-6,8-bis(methylamino)-2,3,4a,5a,6,7,8,9,9a,10a-decahydro-4*H*-pyrano[2,3-*b*][1,4]benzodioxin-4-one dihydrochloride pentahydrate [22189-32-8]

Spectinomycin Hydrochloride Hydrate is the hydrochloride of a substance having antibacterial activity produced by the growth of *Streptomyces spectabilis*.

It contains not less than $603 \,\mu g$ (potency) and not more than 713 μg (potency) per mg. The potency of Spectinomycin Hydrochloride Hydrate is expressed as mass (potency) of spectinomycin (C₁₄H₂₄N₂O₇: 332.35).

Description Spectinomycin Hydrochloride Hydrate occurs as a white to light yellowish white crystalline powder.

It 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 gently anthrone TS: a blue to blue-green color is produced at the zone of contact.

(2) Determine the infrared absorption spectra of Spectinomycin Hydrochloride Hydrate and Spectinomycin Hydrochloride RS as directed in the paste method under the Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare these spectra: 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.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_D^{20}$: +15 - +21° (2.1 g calculated on the anhydrous basis, water, 25 mL, 200 mm).

pH $\langle 2.54 \rangle$ Dissolve 0.10 g of Spectinomycin Hydrochloride Hydrate in 10 mL of water: the pH of the solution is between 4.0 and 5.6.

Water $\langle 2.48 \rangle$ Not less than 16.0% and not more than 20.0% (0.3 g, volumetric titration, direct titration).

Residue on ignition $\langle 2.44 \rangle$ Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

- (i) Test organism-Klebsiella pneumoniae ATCC 10031
- (ii) Culture medium—Use the medium i in 3) under (1)

Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.8 to 8.0 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Spectinomycin Hydrochloride RS, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, 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 not exceeding 5°C and use within 10 days. Take exactly a suitable amount of the standard stock solution, pH 8.0 to make solutions so that each mL contains 200 μ g (potency) and 50 μ g (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Spectinomycin Hydrochloride Hydrate, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 20 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 200 μ g (potency) and 50 μ g (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Spiramycin Acetate

スピラマイシン酢酸エステル



(Spiramycin II Acetate (Spiramycin I Acetate)) (3R,4R,5S,6R,8R,9R,10E,12E,15R)-3-Acetoxy-5-[4-O-acetyl-2,6-dideoxy-3-C-methyl-α-L-*ribo*-hexopyranosyl-(1 \rightarrow 4)-3,6-dideoxy-3dimethylamino-β-D-glucopyranosyloxy]-9-(2,3,4,6tetradeoxy-4-dimethylamino-β-D-erythrohexopyranosyloxy)-6-formylmethyl-9-hydroxy-4-methoxy-8-methylhexadeca-10,12-dien-15-olide (Spiramycin III Acetate) (3R,4R,5S,6R,8R,9R,10E,12E,15R)-5-[4-O-Acetyl-2,6-dideoxy-3-C-methyl-α-L-ribohexopyranosyl- $(1\rightarrow 4)$ -3,6-dideoxy-3-dimethylamino- β -D-glucopyranosyloxy]-9-(2,3,4,6-tetradeoxy-4dimethylamino-*β*-D-erythro-hexopyranosyloxy)-6formylmethyl-9-hydroxy-4-methoxy-8-methyl-3propanoyloxyhexadeca-10,12-dien-15-olide [74014-51-0, Spiramycin Acetate]

Spiramycin Acetate is a derivative of a mixture of macrolide substances having antibacterial activity produced by the growth of *Streptomyces ambofaciens*.

It contains not less than $900 \,\mu\text{g}$ (potency) and not more than $1450 \,\mu\text{g}$ (potency) per mg, calculated on the dried basis. The potency of Spiramycin Acetate is expressed as mass (potency) of spiramycin II acetate (C₄₇H₇₈N₂O₁₆: 927.13). One mg (potency) of Spiramycin Acetate is equivalent to 0.7225 mg of spiramycin II acetate (C₄₇H₇₈N₂O₁₆).

Description Spiramycin Acetate occurs as a white to light yellowish white powder.

It is very soluble in acetonitrile and in methanol, freely soluble in ethanol (99.5), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Spiramycin Acetate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Spiramycin Acetate as directed in the potassium bromide

disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Content ratio of the active principle Dissolve 25 mg of Spiramycin Acetate in 25 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 5 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the areas, A_{II} , A_{III} , A_{IV} , A_V , A_{VI} and A_{VII} , of the peaks of spiramycin II acetate, spiramycin III acetate, spiramycin IV acetate, spiramycin V acetate, spiramycin VI acetate and spiramycin VII acetate, respectively, by the automatic integration method, and calculate the ratios of the amounts of $A_{\rm II}$, $A_{\rm IV}$ and the total of $A_{\rm III}$ and $A_{\rm V}$ to the total amount of all these peaks: the amount of $A_{\rm II}$ is 30 – 45%, $A_{\rm IV}$ is 30 – 45%, and the total of $A_{\rm III}$ and $A_{\rm V}$ is not more than 25%. The relative retention times of spiramycin III acetate, spiramycin IV acetate, spiramycin V acetate, spiramycin VI acetate and spiramycin VII acetate with respect to spiramycin II acetate are 1.3, 1.7, 2.3, 0.85 and 1.4, respectively.

Operating conditions—

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

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μ m in particle diameter).

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

Mobile phase: A mixture of acetonitrile, 0.02 mol/L potassium dihydrogen phosphate TS and a solution of dipotassium hydrogen phosphate (87 in 25,000) (26:7:7).

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

System suitability-

System performance: Dissolve 25 mg of Spiramycin II Acetate RS in the mobile phase to make 100 mL. When the procedure is run with 5μ L of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of spiramycin II acetate are not less than 14,500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with $5 \mu L$ of the sample solution under the above operating conditions, the relative standard deviation of the peak area of spiramycin II acetate is not more than 2.0%.

Purity (1) Heavy metals $\langle 1.07 \rangle$ —Proceed with 1.0 g of Spiramycin Acetate 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 $\langle 1.11 \rangle$ —Prepare the test solution with 2.0 g of Spiramycin Acetate according to Method 3, and perform the test (not more than 1 ppm).

Loss on drying $\langle 2.41 \rangle$ Not more than 3.0% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.5% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics $\langle 4.02 \rangle$ according to the following conditions.

(i) Test organism—Bacillus subtilis ATCC 6633

(ii) Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Spiramycin II Acetate RS, equivalent to about 50 mg (potency), dissolve in 20 mL of methanol, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C, and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains $80 \,\mu g$ (potency) and $20 \,\mu g$ (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Spiramycin Acetate, equivalent to about 50 mg (potency), dissolve in 20 mL of methanol, and add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 80 μ g (potency) and 20 μ g (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Spironolactone

スピロノラクトン



C24H32O4S: 416.57

 7α -Acetylsulfanyl-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 $C_{24}H_{32}O_4S$.

Description Spironolactone occurs as a white to light yellow-brown, fine powder.

It is freely soluble in chloroform, soluble in ethanol (95), slightly soluble in methanol, and practically insoluble in water.

Melting point: $198 - 207^{\circ}$ C (Insert the capillary tube into a bath at about 125° C, and 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 spectrum of a solution of Spironolactone in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Spironolactone RS prepared in

the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Spironolactone, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Spironolactone RS: both spectra exhibit similar intensities of absorption at the same wave numbers. 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.

Optical rotation $\langle 2.49 \rangle$ [α]_D²⁰: $-33 - -37^{\circ}$ (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 develops.

(2) Related substances—Dissolve 0.20 g of Spironolactone in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample 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 Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 5 μ L each of the sample solution and 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: the spots other than the principal spot from the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition $\langle 2.44 \rangle$ 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, dissolve in methanol to make exactly 250 mL. Pipet 5 mL each of these solutions, add methanol to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and determine the absorbances, A_T and A_S , of the sample solution and standard solution at 238 nm.

Amount (mg) of
$$C_{24}H_{32}O_4S$$

= $M_S \times A_T/A_S$

 $M_{\rm S}$: amount (mg) of Spironolactone RS

Containers and storage Containers—Tight containers.

Spironolactone Tablets

スピロノラクトン錠

Spironolactone Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of spironolactone ($C_{24}H_{32}O_4S$: 416.57).

Method of preparation Prepare as directed under Tablets,

with Spironolactone.

Identification To an amount of powdered Spironolactone Tablets, equivalent to 10 mg of Spironolactone according to the labeled amount, add 100 mL of methanol, shake vigorously, and centrifuge. To 5 mL of the supernatant liquid add methanol to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultravioletvisible Spectrophotometry <2.24>: it exhibits a maximum between 236 nm and 240 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Spironolactone Tablets add a mixture of water and acetonitrile (1:1) to make exactly V mL so that each mL contains about 0.5 mg of spironolactone (C₂₄H₃₂O₄S). After stirring for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of spironolactone (
$$C_{24}H_{32}O_4S$$
)
= $M_S \times A_T/A_S \times V/50$

 $M_{\rm S}$: Amount (mg) of Spironolactone RS

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of a solution prepared by dissolving 1 g of polysorbate 80 in water to make 500 mL as the dissolution medium, the dissolution rate in 30 minutes of a 25-mg tablet and a 50-mg tablet are not less than 80% and not less than 70%, respectively.

Start the test with 1 tablet of Spironolactone Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding $0.45 \,\mu\text{m}$. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about $14 \,\mu g$ of spironolactone (C24H32O4S) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Spironolactone RS, previously dried at 105°C for 2 hours, dissolve in 20 mL of ethanol (95), and add the dissolution medium to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, $A_{\rm T}$ and $A_{\rm S}$, at 243 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using the dissolution medium as the blank.

Dissolution rate (%) with respect to the labeled amount of spironolactone $(C_{24}H_{32}O_4S)$

 $= M_{\rm S} \times A_{\rm T}/A_{\rm S} \times V'/V \times 1/C \times 45$

M_S: Amount (mg) of Spironolactone RS

C: Labeled amount (mg) of spironolactone ($C_{24}H_{32}O_4S$) in 1 tablet

Assay Weigh accurately the mass of not less than 10 Spironolactone Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of spironolactone ($C_{24}H_{32}O_4S$), add a mixture of water and acetonitrile (1:1) to make exactly 100 mL. After stirring this solution for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately

about 25 mg of Spironolactone RS, previously dried at 105° C for 2 hours, dissolve in 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 exactly $10 \,\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of spironolactone from each solution.

Amount (mg) of spironolactone (C₂₄H₃₂O₄S) = $M_{\rm S} \times A_{\rm T}/A_{\rm S} \times 2$

 $M_{\rm S}$: Amount (mg) of Spironolactone RS

Operating conditions—

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

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

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

Mobile phase: A mixture of methanol and water (3:2).

Flow rate: Adjust the flow rate so that the retention time of spironolactone is about 11 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of spironolactone are not less than 4000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with $10 \,\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of spironolactone is not more than 1.0%.

Containers and storage Containers—Tight containers.

Stearic Acid

ステアリン酸

Stearic Acid is solid fatty acids obtained from fats, and it consists chiefly of stearic acid ($C_{18}H_{36}O_2$: 284.48) and palmitic acid ($C_{16}H_{32}O_2$: 256.42).

Description Stearic Acid occurs as white, unctuous or crystalline masses or powder. It has a faint, fatty odor.

It is freely soluble in diethyl ether, soluble in ethanol (95), and practically insoluble in water.

Melting point: 56 – 72°C

Acid value <1.13> 194 – 210

Iodine value <1.13> Not more than 4.0.

Purity (1) Mineral acid—Melt 5 g of Stearic Acid by warming, shake with 5 mL of boiling water for 2 minutes, filter after cooling, and add 1 drop of methyl orange TS to the filtrate: no red color develops.

(2) Heavy metals $\langle 1.07 \rangle$ —Proceed with 1.0 g of Stearic 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) Fat and paraffin-Boil 1.0 g of Stearic Acid with

0.5 g of anhydrous sodium carbonate and 30 mL of water: the solution, while hot, is clear or not more turbid than the following control solution.

Control solution: To 0.70 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 30 mL, and add 1 mL of silver nitrate TS.

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Containers and storage Containers-Well-closed containers.

Stearyl Alcohol

ステアリルアルコール

Stearyl Alcohol is a mixture of solid alcohols, and consists chiefly of stearyl alcohol ($C_{18}H_{38}O$: 270.49).

Description Stearyl Alcohol occurs as a white, unctuous matter. It has a faint, characteristic odor. It is tasteless.

It is freely soluble in ethanol (95), in ethanol (99.5), in diethyl ether, and practically insoluble in water.

Melting point <1.13> 56 - 62°C under Melting Point Determination Prepare the sample according to Method 2 under Melting Point Determination, then attach tightly a capillary tube to the bottom of the thermometer by means of a rubber band or by any suitable means, and make the bottom of the capillary tube equal in position to the lower end of the thermometer. Insert this thermometer into a test tube 17 mm in inside diameter and about 170 mm in height, fasten the thermometer with cork stopper so that the lower end of the thermometer is about 25 mm distant from the bottom of the test tube. Suspend the test tube in a beaker containing water, and heat the beaker with constant stirring until the temperature rises to 5°C below the expected melting point. Then regulate the rate of increase to 1°C per minute. The temperature at which the sample is transparent and no turbidity is produced is taken as the melting point.

Acid value <1.13> Not more than 1.0.

Ester value <1.13> Not more than 3.0.

Hydroxyl value <1.13> 200 - 220

Iodine value <1.13> Not more than 2.0.

Purity (1) Clarity of solution—Dissolve 3.0 g of Stearyl Alcohol in 25 mL of ethanol (99.5) by warming: the solution is clear.

(2) Alkalinity—To the solution obtained in (1) add 2 drops of phenolphthalein TS: no red color develops.

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.05% (2 g).

Containers and storage Containers-Well-closed containers.

Streptomycin Sulfate

ストレプトマイシン硫酸塩



 $(C_{21}H_{39}N_7O_{12})_2.3H_2SO_4$: 1457.38 2-Deoxy-2-methylamino- α -L-glucopyranosyl- $(1\rightarrow 2)$ -5-deoxy-3-*C*-formyl- α -L-lyxofuranosyl- $(1\rightarrow 4)$ -*N*, *N'*diamidino-D-streptamine sesquisulfate [3810-74-0]

Streptomycin Sulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of *Streptomyces griseus*.

It contains not less than 740 μ g (potency) and not more than 820 μ g (potency) per mg, calculated on the dried basis. The potency of Streptomycin Sulfate is expressed as mass (potency) of streptomycin (C₂₁H₃₉N₇O₁₂: 581.57).

Description Streptomycin Sulfate occurs as a white to light yellowish white powder.

It 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 is developed.

(2) Dissolve 10 mg each of Streptomycin Sulfate and Streptomycin Sulfate RS in 10 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thinlayer Chromatography $\langle 2.03 \rangle$. Spot 10 μ L each of the sample solution and standard solution 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 from the sample solution and the standard solution show the same in color tone and Rf value.

(3) A solution of Streptomycin Sulfate (1 in 5) responds to the Qualitative Tests $\langle 1.09 \rangle$ for sulfate.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_{D}^{20}$: $-79 - -88^{\circ}$ (0.5 g calculated on the dried basis, water, 50 mL, 100 mm).

pH $\langle 2.54 \rangle$ The pH of a solution obtained by dissolving 2.0 g of Streptomycin Sulfate in 10 mL of water is between 4.5 and 7.0.

Purity (1) Clarity and color of solution—A solution obtained by dissolving 1.0 g of Streptomycin Sulfate in 5 mL of water is clear, and colorless or pale yellow.

(2) Heavy metals $\langle 1.07 \rangle$ —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 $\langle 1.11 \rangle$ —Prepare the test solution with 2.0 g of Streptomycin Sulfate according to Method 3 and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve exactly 0.20 g of Streptomycin Sulfate 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 sample solution. Separately, dissolve exactly 36 mg of D(+)-mannose 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), and add a mixture of methanol and sulfuric acid (97:3) to make exactly 50 mL. Pipet 5 mL of this solution, 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 $\langle 2.03 \rangle$. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thinlayer chromatography, develop 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-dihydroxynaphthalene in ethanol (95) (1 in 500) and diluted sulfuric acid (1 in 5) (1:1) on the plate, and heat at 110°C for 5 minutes: the spot from the sample solution corresponding to the spot from the standard solution is not more intense than the spot from the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 5.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—Bacillus subtilis ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer, having pH 7.8 – 8.0 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Streptomycin Sulfate RS, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer solution, 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 between 5° C and 15° C, and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solu-

tion, pH 8.0 to make solutions so that each mL contains $8 \mu g$ (potency) and $2 \mu g$ (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Streptomycin Sulfate, equivalent to about 20 mg (potency), dissolve in water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 8 μ g (potency) and 2 μ g (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Streptomycin Sulfate for Injection

注射用ストレプトマイシン硫酸塩

Streptomycin Sulfate for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled potency 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 occurs as a white or light yellowish white masses or powder.

Identification Peform the test as directed in the Identification (2) under Streptomycin Sulfate.

Osmotic pressure ratio Being specified separately.

pH $\langle 2.54 \rangle$ The pH of a solution prepared by dissolving an amount of Streptomycin Sulfate for Injection, equivalent to 2.0 g (potency) of Streptomycin Sulfate according to the labeled amount, in 10 mL of water is 5.0 to 7.0.

Purity Clarity and color 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 at 400 nm, determined as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, is not more than 0.50.

Loss on drying $\langle 2.41 \rangle$ Not more than 4.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Bacterial endotoxins <4.01> Less than 0.10 EU/mg (potency).

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Foreign insoluble matter $\langle 6.06 \rangle$ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Perform the test according to the Cylinder-plate

JP XVI

method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organisms, culture medium and standard solutions—Proceed as directed in the Assay under Streptomycin Sulfate.

(ii) Sample solution—Weigh acculately the contents of not less than 10 Streptomycin Sulfate for Injection. Weigh accurately an amount of the contents, equivalent to 1 g (potency) of Streptomycin Sulfate, and dissolve in water to make exactly 200 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0, to make a solutions so that each mL contains 8 μ g (potency) and 2 μ g (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Hermetic containers.

Sucralfate Hydrate

Aluminum Sucrose Sulfate Ester





 $C_{12}H_{30}Al_8O_{51}S_8.xAl(OH)_3.yH_2O$ [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 occurs as a white powder. It is odorless and tasteless.

It is practically insoluble in water, in hot water, in ethanol (95) and in diethyl ether.

It dissolves in dilute hydrochloric acid and in sulfuric acidsodium hydroxide TS.

Identification (1) To 0.05 g of Sucralfate Hydrate in a small test tube add 0.05 g of fresh pieces of 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 develops.

(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 $\langle 1.09 \rangle$ 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 <1.03>—Dissolve 0.5 g of Sucralfate Hy-

drate 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 using 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 $\langle 1.07 \rangle$ —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 using 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 20 ppm).

(4) Arsenic $\langle 1.11 \rangle$ —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).

(5) 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 portions 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 sample solution. Pipet 50 mL of the sample solution, add exactly 25 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate 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 <2.50> the excess disodium dihydrogen ethylenediamine tetraacetate 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 (not more than 0.2%).

> Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 1.349 mg of Al

(6) Related substances—Proceed with 50 μ L of the sample solution obtained in the Assay (2) Sucrose octasulfate ester as directed in the Assay (2) Sucrose octasulfate ester, and perform the test as directed under Liquid Chromatography $\langle 2.0I \rangle$. Determine the peak area of sucrose octasulfate ester from the sample 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 $50 \,\mu\text{L}$ of the standard solution obtained in the Assay (2) Sucrose octasulfate ester composes 60% to 100% of the full scale.

Loss on drying $\langle 2.41 \rangle$ 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, place in a 200-mL glass-stoppered conical flask, add exactly 100 mL of 0.1 mol/L hydrochloric acid VS, stopper the flask tightly, and shake at $37 \pm 2^{\circ}$ C for exactly 1 hour (150 shakings per

1430 Sucrose / Official Monographs

minute, amplitude: 20 mm). After cooling in water for 5 minutes, pipet 10 mL of the supernatant liquid, and titrate $\langle 2.50 \rangle$ the excess acid with 0.1 mol/L sodium hydroxide VS until the pH becomes 3.5. Perform a blank determination. The amount of 0.1 mol/L hydrochloric acid VS consumed per g of Sucralfate 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 dihydrogen ethylene-diamine tetraacetate 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 $\langle 2.50 \rangle$ the excess disodium dihydrogen ethylenediamine tetraacetate 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.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 1.349 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 sample 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 sample solution and the standard solution, and perform the test immediately. Pipet 50 μ L each of the sample solution and standard solution, and perform the test as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of sucrose octasulfate ester from each solution.

- Amount (mg) of sucrose octasulfate ester $(C_{12}H_{22}O_{35}S_8)$ = $M_S \times A_T/A_S \times 0.763$
- $M_{\rm S}$: Amount (mg) of Potassium Sucrose Octasulfate RS, calculated on the anhydrous basis

Operating conditions—

Detector: A differential refractometer.

Column: A stainless steel column about 4 mm in inside diameter and about 30 cm in length, packed with aminopropylsilanized silica gel for liquid chromatography (about 8 μ m in particle diameter).

Column temperature: Room temperature.

Mobile phase: Dissolve a suitable amount (26 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 returns 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.

Selection of column: 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 proceed immediately with 50 μ L of this solution under the above operating conditions. Use a column with a resolution being not less than 1.5 between sucrose octasulfate ester and a related substance with the relative retention time being about 0.7 to sucrose octasulfate ester.

System repeatability: Repeat the test 6 times with 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.

Sucrose

精製白糖



 $C_{12}H_{22}O_{11}$: 342.30 β -D-Fructofuranosyl α -D-glucopyranoside [57-50-1]

Sucrose contains no additives.

For Sucrose used for preparation of the large volume infusions, the label states the purpose.

Description Sucrose is a white crystalline powder, or lustrous colorless or white crystals.

It is very soluble in water, and slightly soluble in ethanol (95).

Identification (1) To 10 mg each of Sucrose and white soft sugar add diluted methanol (3 in 5) to make 20 mL each, and use these solutions as the sample solution and the standard solution (a), respectively. Separately, to 10 mg each of glucose, lactose monohydrate, fructose and white soft sugar add methanol (3 in 5) to make 20 mL, and use this solution as the standard solution (b). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μ L each of the sample solution and standard solutions (a) and (b) on a plate of silica gel for thin-layer chromatography, and dry the plate completely. Develop the plate with a mixture of 1,2-dichloroethane, acetic acid (100), methanol and water (10:5:3:2) to a distance of about 15 cm, and dry the plate with a hot air. And immediately repeat the development with replaced developing mixture, and dry the plate in the same way. Spray evenly a solution of 0.5 g of thymol in 100 mL of a mixture of ethanol (95) and sulfuric acid (19:1), heat at 130°C for 10 minutes: the principal spot from the sample solution is the same with the principal spot from the standard solution (a) in the Rf value, color and size, and four spots from the standard solution (b) are apparently distinguishable.

(2) Dissolve 50.0 g of Sucrose in recently boiled and cooled water to make 100 mL, and use this solution as the sample solution. To 1 mL of the sample solution add water to make 100 mL, then to 5 mL of this solution add 0.15 mL of freshly prepared copper (II) sulfate TS and 2 mL of freshly prepared 2 mol/L sodium hydroxide TS: the solution is clear and blue, and not changes on boiling. Then to this solution add 4 mL of 2 mol/L sodium hydroxide TS: orange precipitates are immediately produced.

Optical rotation $\langle 2.49 \rangle$ [α]_D²⁰: +66.3 - +67.0° (26 g, water, 100 mL, 100 mm).

Purity (1) Clarity and color of solution—The sample solution obtained in the Identification (2) is clear, and has no more color than the following control solution.

Control solution: To exactly 2.4 mL of Iron (III) Chloride CS and exactly 0.6 mL of Cobalt (II) Chloride CS add 7.0 mL of diluted hydrochloric acid (7 in 250). To 5.0 mL of this solution add 95.0 mL of diluted hydrochloric acid (7 in 250).

(2) Acidity or alkalinity—To 10 mL of the sample solution obtained in the Identification (2) add 0.3 mL of phenolphthalein TS: the solution is colorless, and develops a red color on addition of 0.3 mL of 0.01 mol/L sodium hydroxide TS.

(3) Sulfite—Dissolve 5.0 g of Sucrose in 40 mL of water, add 2.0 mL of dilute sodium hydroxide TS and water to make exactly 50 mL, and use this solution as the sample solution. Separately, dissolve 76 mg of sodium disulfite in water to make exactly 50 mL, then pipet 5 mL of this solution, add water to make exactly 100 mL. Pipet 3 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Immediately, pipet 10 mL each of the sample solution and the standard solution, add 1.0 mL of 3 mol/L hydrochloric acid TS, 2.0 mL of decolorized fuchsin TS and 2.0 mL of formaldehyde solution TS, and allow to stand for 30 minutes. Determine the absorbance at 583 nm of these solutions as directed under Ultravioletvisible Spectrophotometry <2.24> using the control solution obtained by proceeding with 10.0 mL of water in the same manner as above: the absorbance of the sample solution is not larger than that of the standard solution (not more than 15 ppm as SO_2). When the standard solution does not show a red-purple to blue-purple color, result of the test is invalid.

(4) Lead—Put exactly 50 mg of Sucrose in a polytetrafuruoroethylene decomposition-vessel, add 0.5 mL of nitric acid to dissolve, seal up the vessel, and heat at 150° C for 5 hours. After cooling, add water to make exactly 5 mL, and use this solution as the sample solution. Perform the test with more than 3 parts of the sample solution as directed in the standard addition method under Atomic Absorption Spectrophotometry $\langle 2.23 \rangle$ (electrothermal type) according to the following conditions. The standard solution is prepared by adding water to a suitable volume of Standard Lead Solution exactly volumed, and perform a blank determination with a solution prepared by adding water to 10.0 mL of nitric acid to make exactly 100 mL, and make any necessary correction (not more than 0.5 ppm).

Operating conditions—

Lamp: A hollow cathode lamp. Wavelengh: 283.3 mm. Temperature for drying: 110°C.

Temperature for incineration: 600°C.

Temperature for atomization: 2100°C.

(5) Invert sugar—Transfer 5 mL of the sample solution obtained in the Identification (2) to a test-tube about 150 mm long and about 16 mm in diameter, add 5 mL of water, 1.0 mL of 1 mol/L sodium hydroxide VS and 1.0 mL of methylene blue TS, mix, and place in a water bath. After exactly 2 minutes, take the tube out of the bath, and examine the solution immediately: the blue color does not disappear completely (0.04%). Ignore any blue color at the air and solution interface.

Conductivity

(i) Potassium chloride conductivity calibration standard solution—Dissolve powdered potassium chloride, previously dried at 500 – 600°C for 4 hours, in newly distillated water having less conductivity than $2 \,\mu \text{S} \cdot \text{cm}^{-1}$ to get three kinds of the standard solution containing 0.7455 g, 0.0746 g and 0.0149 g of potassium chloride in 1000.0 g, respectively. The conductivities of these solutions at 20°C are shown in the following table.

Standard solution (g/1000.0 g)	Conductivity $(\mu S \cdot cm^{-1})$	Resistivity (Ω · cm)	
0.7455	1330	752	
0.0746	133.0	7519	
0.0149	26.6	37594	

(ii) Apparatus—Use an appropriate conductivity meter. The conductivity is determined to measure the electrical resistance of the column of liquid between the electrodes of the immersed measuring device (conductivity cell). The apparatus is supplied with alternative current to avoid the effects of electrode polarization. It is usually equipped with a temperature compensation device. The conductivity cell contains of two parallel platinum electrodes coated with platinum black, and both electrodes are generally protected by a glass tube which allows good exchange between the solution and the electrodes. Use a cell giving the cell constant of 0.01 to 1 cm^{-1} .

(iii) Procedure—Use the suitable potassium chloride conductivity calibration standard solution to the measurement. After washing the well with water, rinse 2 to 3 times with the calibration standard solution, fill up the cell with the calibration standard solution, and determine the conductivity of the calibration standard solution kept at 20 \pm 0.1°C. Repeat the determination, and measure the conductivity of the calibration standard solution, G_{χ_0} (μ S), after a stable reading of \pm 3% is obtained. The cell constant, *J*, is calculated by the following:

$$J = \frac{\chi_{\rm KCl}}{G_{\chi_0}}$$

J: Cell constant (cm^{-1})

 χ_{KCl} : Conductivity constant of the potassium chloride conductivity calibration standard solution (μ S·cm⁻¹) (20°C)

 G_{χ_0} : Conductivity measured (μ S)

Dissolve 31.3 g of Sucrose in newly distillated water to make exactly 100 mL, and use this solution as the sample so-

lution. After washing well the cell with water, rinse the cell with the sample solution 2 to 3 times, fill up with the sample solution, and determine the conductivity of the sample solution, $G_{\rm T}$ (μ S), kept at 20 ± 0.1 °C, while stirring. Determine the conductivity of the water used for preparation of the sample solution, G_0 (μ S), in the same manner as above, and calculate the conductivity, $\chi_{\rm T}$ (μ S·cm⁻¹) and χ_0 (μ S·cm⁻¹), by the following expressions:

$$\chi_{\mathrm{T}} (\mu \mathrm{S} \cdot \mathrm{cm}^{-1}) = JG_{\mathrm{T}}$$

$$\chi_{0} (\mu \mathrm{S} \cdot \mathrm{cm}^{-1}) = JG_{0}$$

Determine the corrected conductivity, χ_c , of the sample solution by the following expression: not more than 35 μ S·cm⁻¹.

$$\chi_{\rm C} (\mu {\rm S} \cdot {\rm cm}^{-1}) = \chi_{\rm T} - 0.35 \, \chi_0$$

Loss on drying $\langle 2.41 \rangle$ Not more than 0.1% (2 g, 105°C, 3 hours).

Dextrins For Sucrose used to prepare large volume infusions, to 2 mL of the sample solution obtained in the Identification (2) add 8 mL of water, 0.05 mL of dilute hydrochloric acid and 0.05 mL of iodine TS: the solution remains yellow.

Bacterial endotoxins <4.01> Less than 0.25 EU/mg, for Sucrose exclusively to be used to prepare Injections for intravenous infusion of larger volume.

Containers and storage Containers-Well-closed containers.

White Soft Sugar

白糖



 $C_{12}H_{22}O_{11}$: 342.30 β -D-Fructofuranosyl α -D-glucopyranoside [57-50-1]

Description White Soft Sugar is colorless or white crystals or crystalline powder. It is odorless and has a sweet taste.

It is very soluble in water, very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

A solution of White Soft Sugar (1 in 10) is neutral.

Identification (1) When 1 g of White Soft Sugar is ignited, it melts and swells, and decomposes, emitting an odor of caramel, to bulky charcoal.

(2) To 0.1 g of White Soft Sugar add 2 mL of dilute sulfuric acid, boil, add 4 mL of sodium hydroxide TS and 3 mL of Fehling's TS, and heat to boiling: a red to dark red precipitate is produced.

Optical rotation $\langle 2.49 \rangle$ [α]_D²⁰: +65.0 - +67.0° (after dry-

ing, 13 g, water, 50 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 100 g of White Soft Sugar in 100 mL of water, take 50 mL of this solution in a Nessler tube, and view transversely the Nessler tube against a white background: the solution is colorless or only slightly yellow and has no blue color. Fill the solution in the Nessler tube, stopper, and allow to stand for 2 days: no precipitate is produced.

(2) Chloride $\langle 1.03 \rangle$ —To 10.0 g of White Soft Sugar add water to make 100 mL, and use this solution as the sample solution. To 20 mL of the sample 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 with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.005%).

(3) Sulfate $\langle 1.14 \rangle$ —To 40 mL of the sample solution obtained in (2) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Propare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.006%).

(4) Calcium—To 10 mL of the sample solution obtained in (2) add 1 mL of ammonium oxalate TS: this solution shows immediately no change.

(5) Heavy metals $\langle 1.07 \rangle$ —Proceed with 5.0 g of White Soft Sugar 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) Arsenic $\langle 1.11 \rangle$ —Prepare the test solution with 1.0 g of White Soft Sugar according to Method 1, and perform the test (not more than 2 ppm).

(7) Invert sugar—Dissolve 5.0 g of White Soft Sugar in water to make 100 mL, filter if necessary, and use this solution as the sample solution. Separately place 100 mL of alkaline copper (II) sulfate solution in a 300-mL beaker, cover the beaker with a watch glass, and boil. Immediately add 50.0 mL of the sample solution, boil the mixture exactly for 5 minutes, add at once 50 mL of freshly boiled and cooled water, dip it in a water bath of a temperature below 10°C for 5 minutes, and collect the precipitate in a tared glass filter (G4). Wash the residue on the filter with water until the last washing is neutral, then wash with 10 mL of ethanol (95), add 10 mL of diethyl ether, and dry at 105°C for 30 minutes: the mass of the residual precipitate is not more than 0.120 g.

Loss on drying $\langle 2.41 \rangle$ Not more than 1.30% (15 g, 105°C, 2 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (2 g).

Containers and storage Containers—Well-closed containers.
Sulbactam Sodium

スルバクタムナトリウム

C₈H₁₀NNaO₅S: 255.22

Monosodium (2*S*,5*R*)-3,3-dimethyl-7-oxo-4-thia-1azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide [*69388-84-7*]

Sulbactam Sodium contains not less than $875 \,\mu g$ (potency) and not more than $941 \,\mu g$ (potency) per mg, calculated on the anhydrous basis. The potency of Sulbactam Sodium is expressed as mass (potency) of sulbactam (C₈H₁₁NO₅S: 233.24).

Description Sulbactam Sodium occurs as a white to yellowish white crystalline powder.

It 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 absorption spectrum of Sulbactam Sodium as directed in the potassium bromide disk method under the Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Sulbactam Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_{D}^{20}$: $+219 - +233^{\circ}$ (1 g, water, 100 mL, 100 mm).

pH $\langle 2.54 \rangle$ Dissolve 1.0 g of Sulbactam Sodium in 20 mL of water: the pH of the solution 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 <1.07>—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 $\langle 1.11 \rangle$ —Prepare the test solution with 1.0 g of Sulbactam Sodium as directed in 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 sample 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 for 10 minutes at a room temperature, and add 0.5 mL of 1 mol/L hydrochloric acid TS, then 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 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$,

of subactam penicillamine by the automatic integration method: the amount of subactam penicillamine is not more than 1.0%.

Amount (%) of sulbactam penicillamine = $M_S/M_T \times A_T/A_S \times 5$

 $M_{\rm S}$: Amount (mg) of sulbactam sodium for sulbactam penicillamine

 $M_{\rm T}$: Amount (mg) of the sample

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 (wave-length: 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 $10 \,\mu$ L 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 $\langle 2.48 \rangle$ Not more than 1.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Sulbactam Sodium and Sulbactam RS, equivalent to about 0.1 g (potency), dissolve each in a suitable amount of the mobile phase, add exactly 10 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of sulbactam to that of the internal standard.

Amount [μ g (potency)] of sulbactam (C₈H₁₁NO₅S) = $M_{\rm S} \times Q_{\rm T}/Q_{\rm S} \times 1000$

M_S: Amount [mg (potency)] of Sulbactam RS

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 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

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

Mobile phase: To 750 mL of 0.005 mol/L tetrabutylammonium hydroxide TS add 250 mL of acetonitrile for liquid chromatography.

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 μ L 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 $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of sublactam is not more than 1.0%.

Containers and storage Containers-Tight containers.

Sulbenicillin Sodium

スルベニシリンナトリウム



 $C_{16}H_{16}N_2Na_2O_7S_2: 458.42$ Disodium (2S,5R,6R)-3,3-dimethyl-7-oxo-6-[(2R)-2-phenyl-2-sulfonatoacetylamino]-4-thia-1azabicyclo[3.2.0]heptane-2-carboxylate [28002-18-8]

Sulbenicillin Sodium contains not less than 900 μ g (potency) and not more than 970 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Sulbenicillin Sodium is expressed as mass (potency) of sulbenicillin (C₁₆H₁₈N₂O₇S₂: 414.45).

Description Sulbenicillin Sodium occurs as white to light yellowish white powder.

It is very soluble in water, freely soluble in methanol, and slightly soluble in ethanol (99.5).

It is hygroscopic.

Identification (1) Determine the infrared absorption spectrum of Sulbenicillin Sodium as directed in the potassium bromide disk method under the Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Sulbenicillin Sodium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Sulbenicillin Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation $\langle 2.49 \rangle$ [α]_D²⁰: +167 – +182° (1 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH $\langle 2.54 \rangle$ 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 $\langle 1.07 \rangle$ —Proceed with 1.0 g of Subenicillin 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 $\langle 1.11 \rangle$ —Prepare the test solution with 1.0 g of Sulbenicillin Sodium according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Sulbenicillin Sodium in 15 mL of the mobile phase, and use this solution as the sample solution. Perform the test with $10 \,\mu$ L of the sample solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of these peaks by the area percentage method: the amount of the each peak other than the two peaks of sulbenicillin is not more than 2.0%, and the total amount 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 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m 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 of subenicillin is about 18 minutes.

Time span of measurement: About 1.5 times as long as the retention time of the lately eluted peak of sulbenicillin beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 1 mL of the sample solution, add the mobile phase 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 to make exactly 10 mL. Confirm that the total area of the two peaks of sulbenicillin obtained from $10 \,\mu$ L of this solution is equivalent to 7 to 13% of that from $10 \,\mu$ L of the solution for system suitability test.

System performance: When the procedure is run with 10 μ L of the sample solution under the above operating conditions, the resolution between the two peaks of subenicillin is not less than 2.0.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ of the solution for system suitability test 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%.

Water $\langle 2.48 \rangle$ Not more than 6.0% (0.5 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics $\langle 4.02 \rangle$ according to the following conditions.

(i) Test organism-Bacillus subtilis ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 6.4 to 6.6 after sterilization.

(iii) Standard solutions—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. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 40 μ g (potency) and 10 μ g (potency), and use these solutions as

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the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—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. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains $40 \,\mu g$ (potency) and $10 \,\mu g$ (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Hermetic containers.

Sulfadiazine Silver

スルファジアジン銀



C₁₀H₉AgN₄O₂S: 357.14 Monosilver 4-amino-*N*-(pyrimidin-2-yl)-benzenesulfonamidate [*22199-08-2*]

Sulfadiazine Silver, when dried, contains not less than 99.0% and not more than 102.0% of $C_{10}H_9AgN_4O_2S$.

Description Sulfadiazine Silver occurs as a white to pale yellow, crystalline powder. It is odorless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in ammonia TS.

It is gradually colored by light.

Melting point: about 275°C (with decomposition).

Identification Determine the infrared absorption spectrum of Sulfadiazine Silver, previously dried, as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Sulfadiazine Silver RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Nitrate—To 250 mL of water add 1.0 g of Sulfadiazine Silver, shake well for 50 minutes, filter, and use this filtrate as the sample solution. Separately, weigh accurately 0.25 g of potassium nitrate, and dissolve in water to make exactly 2000 mL. Pipet 5 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 sample solution and the standard solution, and add 5 mL of a solution of cromotropic acid in sulfuric acid (1 in 10,000) and sulfuric acid to make exactly 10 mL. Determine the absorbances, $A_{\rm T}$ and $A_{\rm S}$, of the sample solution and standard solution at 408 nm as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, using a solution, prepared with exactly 2.0 mL of water in the same manner, as the blank: $A_{\rm T}$ is not larger than $A_{\rm S}$ (not more than 0.05%).

(2) Related substances—Dissolve 50 mg of Sulfadiazine

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Silver in 5 mL of a mixture of ethanol (95) and ammonia solution (28) (3:2), and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add a mixture of ethanol (95) and ammonia solution (28) (3:2) to make exactly 20 mL. Pipet 2 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 these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 5 μ L each of the sample solution and 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 sample solution are not more intense than the spot from the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 80%, 4 hours).

Residue on ignition $\langle 2.44 \rangle$ 41 – 45% (1 g).

Silver content Weigh accurately about 50 mg of Sulfadiazine Silver, 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 sample solution. Measure accurately a suitable quantity of Standard Silver Solution for Atomic Absorption Spectrophotometry, dilute with water to make solutions containing 1.0 to 2.0 μ g of silver (Ag:107.87) per mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions, and calculate the silver content of the sample solution from the calibration curve obtained from the absorbances of the standard solutions: it contains not less than 28.7% and not more than 30.8% of silver.

Gas: Combustible gas—Acetylene. Supporting gas—Air. Lamp: A silver hollow cathode lamp. Wavelength: 328.1 nm.

Assay Weigh accurately about 0.1 g each of Sulfadiazine Silver and Sulfadiazine Silver RS, each previously dried, and add ammonia TS to make exactly 100 mL, respectively. Pipet 1 mL each of these solutions, add water to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 255 nm, as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, using a solution, prepared with exactly 1 mL of ammonia TS and a sufficient water to make exactly 100 mL, as the blank.

> Amount (mg) of $C_{10}H_9AgN_4O_2S$ = $M_S \times A_T/A_S$

M_S: Amount (mg) of Sulfadiazine Silver RS

Containers and storage Containers—Well-closed containers.

Storage-Light-resistant.

Sulfamethizole

スルファメチゾール



 $C_9H_{10}N_4O_2S_2$: 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% of $C_9H_{10}N_4O_2S_2$.

Description Sulfamethizole occurs as white to yellowish white crystals or crystalline powder. It is odorless.

It is slightly soluble in ethanol (95), and in acetic acid (100) and practical insoluble in water and in diethyl ether.

It dissolves in dilute hydrochloric acid and in sodium hydroxide TS.

It is gradually colored by light.

Identification Determine the infrared absorption spectrum of Sulfamethizole, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 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) Acidity—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 develops.

(3) Heavy metals $\langle 1.07 \rangle$ —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 $\langle 1.11 \rangle$ —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 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 50 mL, then pipet 5 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 Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 5 μ L each of the sample 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 acetic acid (100) (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 sample solution are not more intense than the spot from the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 $\langle 2.50 \rangle$ with 0.1 mol/L sodium nitrite VS according to the potentiometric titration method or the amperometric titration method.

> Each mL of 0.1 mol/L sodium nitrite VS = $27.03 \text{ mg of } C_9H_{10}N_4O_2S_2$

Containers and storage Containers—Well-closed containers.

Storage-Light-resistant.

Sulfamethoxazole

Sulfisomezole



 $C_{10}H_{11}N_3O_3S: 253.28$ 4-Amino-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide [723-46-6]

Sulfamethoxazole, when dried, contains not less than 99.0% of $C_{10}H_{11}N_3O_3S$.

Description Sulfamethoxazole occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is very soluble in N,N-dimethylformamide, sparingly soluble in ethanol (95), slightly soluble in diethyl ether, and very slightly soluble in water.

It dissolves in sodium hydroxide TS.

It is gradually colored by light.

Identification Determine the infrared absorption spectrum of Sulfamethoxazole, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 169 – 172°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Sulfamethoxazole in 5 mL of sodium hydroxide TS, and add 20 mL of water: the solution is clear and colorless.

(2) Acidity—To 1.0 g of Sulfamethoxazole add 50 mL of water, heat at 70°C for 5 minutes, allow to stand in ice water 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 VS: a yellow color develops.

(3) Heavy metals $\langle 1.07 \rangle$ —Proceed with 1.0 g of Sul-

famethoxazole 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 $\langle 1.11 \rangle$ —Prepare the test solution with 1.0 g of Sulfamethoxazole according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.20 g of Sulfamethoxazole in 10 mL of a solution of ammonia solution (28) in methanol (1 in 50), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a solution of ammonia solution (28) in methanol (1 in 50) to make exactly 10 mL. Pipet 1 mL of this solution, add a solution of ammonia solution (28) in methanol (1 in 50) 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 $\langle 2.03 \rangle$. Spot 5 μ L each of the sample 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, acetonitrile and diluted ammonia solution (28) (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 sample solution are not more intense than the spot from the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Sulfamethoxazole, previously dried, dissolve in 30 mL of N,N-dimethyl-formamide, add 10 mL of water, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L sodium hydroxide VS until a light blue color is produced (indicator: 0.5 mL of thymolphthalein TS). Separately, perform a blank determination in the same manner with a mixture of 30 mL of N,N-dimethylformamide and 26 mL of water, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 25.33 mg of C₁₀H₁₁N₃O₃S

Containers and storage Containers-Well-closed containers.

Storage—Light-resistant.

Sulfamonomethoxine Hydrate

スルファモノメトキシン水和物



C₁₁H₁₂N₄O₃S.H₂O: 298.32 4-Amino-*N*-(6-methoxypyrimidin-4-yl)benzenesulfonamide Monohydrate [*1220-83-3*, anhydride]

Sulfamonomethoxine Hydrate, when dried, contains not less than 99.0% of sulfamonomethoxine $(C_{11}H_{12}N_4O_3S: 280.31)$. **Description** Sulfamonomethoxine Hydrate occurs as white to pale yellow crystals, granules or crystalline powder. It is odorless.

It is soluble in acetone, slightly soluble in ethanol (95), very slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid and in sodium hydroxide TS.

It is gradually colored by light.

Identification Determine the infrared absorption spectrum of Sulfamonomethoxine Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 204 – 206°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Sulfamonomethoxine Hydrate in 5 mL of sodium hydroxide TS and 20 mL of water: the solution is clear and colorless to pale yellow. Dissolve 0.5 g of Sulfamonomethoxine Hydrate in 5 mL of sodium hydroxide TS, and heat: no turbidity is produced. After cooling, add 5 mL of acetone: the solution is clear.

(2) Heavy metals $\langle 1.07 \rangle$ —Proceed with 1.0 g of Sulfamonomethoxine 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).

(3) Arsenic $\langle 1.11 \rangle$ —Prepare the test solution with 1.0 g of Sulfamonomethoxine Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.02 g of Sulfamonomethoxine Hydrate in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) 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 $\langle 2.03 \rangle$. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol and ammonia solution (28) (4:1) 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 sample solution are not larger and not more intense than the spot from the standard solution.

Loss on drying <2.41> 4.5 – 6.5% (1 g, 105°C, 4 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.10% (1 g).

Assay Weigh accurately about 0.5 g of Sulfamonomethoxine Hydrate, 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 $\langle 2.50 \rangle$ with 0.1 mol/L sodium nitrite VS (potentiometric titration or amperometric titration).

> Each mL of 0.1 mol/L sodium nitrite VS = $28.03 \text{ mg of } C_{11}H_{12}N_4O_3S$

Containers and storage Containers-Well-closed containers.

Storage-Light-resistant.

Sulfisoxazole

Sulfafurazole

スルフイソキサゾール



C₁₁H₁₃N₃O₃S: 267.30 4-Amino-*N*-(3,4-dimethylisoxazol-5-yl)benzenesulfonamide [*127-69-5*]

Sulfisoxazole, when dried, contains not less than 99.0% of $C_{11}H_{13}N_3O_3S$.

Description Sulfisoxazole occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in pyridine and in *n*-butylamine, soluble in methanol, sparingly soluble in ethanol (95), slightly soluble in acetic acid (100), and very slightly soluble in water and in diethyl ether.

It dissolves in dilute hydrochloric acid, in sodium hydroxide TS and in ammonia TS.

It 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 <1.09> 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 (II) 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 (II) sulfate TS, and shake. Add 3 mL of water and 5 mL of chloroform, shake, and allow to stand: a light yellow-brown color develops in the chloroform layer.

(4) To 0.5 g of Sulfisoxazole add 2 mL of acetic acid (100), dissolve by heating under a reflux condenser, add 1 mL of acetic anhydride, and boil for 10 minutes. Add 10 mL of water, cool, and alkalize with about 7 mL of a solution of sodium hydroxide (3 in 10). Filter, if necessary, immediately acidify by adding acetic acid (100) dropwise, collect the produced precipitate, recrystallize from methanol, and dry at 105°C for 1 hour: the crystals melt $\langle 2.60 \rangle$ between 208°C and 210°C.

Melting point <2.60> 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) Acidity—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 TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: a yellow color develops.

(3) Heavy metals <1.07>—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).

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (2 g, 105°C, 4 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 $\langle 2.50 \rangle$ with 0.2 mol/L sodium hydroxide VS (indicator: 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 = $53.46 \text{ mg of } C_{11}H_{13}N_3O_3S$

Containers and storage Containers-Well-closed containers.

Storage—Light-resistant.

Sulfobromophthalein Sodium

スルホブロモフタレインナトリウム



 $\begin{array}{l} C_{20}H_8Br_4Na_2O_{10}S_2: 838.00\\ Disodium 5,5'-(4,5,6,7-tetrabromo-3-oxo-1,3-dihydroisobenzofuran-1,1-diyl)bis(2-hydroxybenzenesulfonate)\\ [71-67-0] \end{array}$

Sulfobromophthalein Sodium, when dried, contains not less than 96.0% and not more than 104.0% of $C_{20}H_8Br_4Na_2O_{10}S_2$.

Description Sulfobromophthalein Sodium occurs as a white, crystalline powder. It is odorless.

It is soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It is hygroscopic.

Identification (1) Dissolve 0.02 g of Sulfobromophthalein Sodium in 10 mL of water, and add 1 mL of sodium carbonate TS: a blue-purple color is produced. Add 1 mL of dilute hydrochloric acid to the solution: the color of the solution disappears.

(2) Transfer 0.2 g of Sulfobromophthalein Sodium to a porcelain crucible, mix well with 0.5 g of anhydrous sodium carbonate, and ignite until the mixture is charred. After cooling, add 15 mL of hot water to the residue, heat for 5 minutes on a water bath, filter, and render the filtrate slightly acid with hydrochloric acid: the solution responds to the Qualitative Tests $\langle 1.09 \rangle$ for bromide, and (1) and (2) for sulfate.

(3) Sulfobromophthalein Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt. **pH** <2.54> The pH of a solution of 1.0 g of Sulfobromophthalein Sodium in 20 mL of water is between 4.0 and 5.5.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Sulfobromophthalein Sodium in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Chloride $\langle 1.03 \rangle$ —Perform the test with 2.0 g of Sulfobromophthalein Sodium. Prepare the control solution with 0.10 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.002%).

(3) Sulfate—To 10 mL of a solution of Sulfobromophthalein Sodium (1 in 500) add 5 drops of dilute hydrochloric acid, heat to boil, and add 1 mL of hot barium chloride TS: the solution is clear when observed 1 minute after the addition of the barium chloride TS.

(4) Calcium—Weigh accurately about 5 g of Sulfobromophthalein Sodium, transfer to a porcelain dish, heat gently to char, and heat strongly between 700°C and 750°C until the residue is incinerated. After cooling, add 10 mL of dilute hydrochloric acid, and heat for 5 minutes on a water bath. Transfer the contents to a flask with 50 mL of water, and add 5 mL of 8 mol/L potassium hydroxide TS and 0.1 g of NN indicator. Titrate $\langle 2.50 \rangle$ with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the red-purple color of the solution changes to blue.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 0.4008 mg of Ca

The content of calcium (Ca: 40.08) is not more than 0.05%.

(5) Heavy metals $\langle 1.07 \rangle$ —Proceed with 1.0 g of Sulfobromophthalein 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).

(6) Arsenic $\langle 1.11 \rangle$ —Transfer 0.65 g of Sulfobromophthalein Sodium to a crucible, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), fire to burn, then heat gently until the residue is incinerated. If any carbon remains, moisten the residue with a small amount of nitric acid, and incinerate again by ignition. After cooling, add 10 mL of dilute sulfuric acid, and heat until white fumes are evolved. After cooling, add 5 mL of water to the residue, and perform the test with this solution as the test solution (not more than 3.1 ppm).

Loss on drying $\langle 2.41 \rangle$ Not more than 5.0% (0.5 g, 105°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ 14 – 19% (after drying, 0.5 g, 700 – 750°C).

Assay Dissolve about 0.1 g of Sulfobromophthalein Sodium, previously dried and accurately weighed, in water to make exactly 500 mL. Pipet 5 mL of this solution, and add a solution of anhydrous sodium carbonate (1 in 100) to make exactly 200 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$. Determine the absorbance A of this solution at the wavelength of maximum absorption at about 580 nm, using water as the blank.

> Amount (mg) of $C_{20}H_8Br_4Na_2O_{10}S_2$ = $A/881 \times 200,000$

Containers and storage Containers—Tight containers.

Storage-Light-resistant.

Sulfobromophthalein Sodium Injection

スルホブロモフタレインナトリウム注射液

Sulfobromophthalein Sodium Injection is an aqueous solution for Injection.

It contains not less than 94.0% and not more than 106.0% of the labeled amount of sulfobromophthalein sodium ($C_{20}H_8Br_4Na_2O_{10}S_2$: 838.00).

Method of preparation Prepare as directed under Injections, with Sulfobromophthalein Sodium.

Description Sulfobromophthalein Sodium Injection is a clear and colorless or pale yellow liquid.

pH: 5.0 - 6.0

Identification (1) Measure a volume of Sulfobromophthalein Sodium Injection, equivalent to 0.02 g of Sulfobromophthalein Sodium according to the labeled amount, and proceed as directed in the Identification (1) under Sulfobromophthalein Sodium.

(2) Measure a volume of Sulfobromophthalein Sodium Injection, equivalent to 0.1 g of Sulfobromophthalein Sodium according to the labeled amount, add 0.5 g of anhydrous sodium carbonate, and evaporate on a water bath to dryness. Ignite the residue until it is charred. Proceed as directed in the Identification (2) under Sulfobromophthalein Sodium.

Pyrogen $\langle 4.04 \rangle$ Add isotonic sodium chloride solution to Sulfobromophthalein Sodium Injection to make a 0.5 w/v% solution of Sulfobromophthalein Sodium according to the labeled amount. Inject into each of the rabbits 5 mL of this solution per kg of body mass: it meets the requirement.

Extractble volume <6.05> It meets the repequirement.

Assay Measure exactly a volume of Sulfobromophthalein Sodium Injection, equivalent to about 0.1 g of sulfobromophthalein sodium ($C_{20}H_8Br_4Na_2O_{10}S_2$), add water to make exactly 500 mL, and proceed as directed in the Assay under Sulfobromophthalein Sodium.

> Amount (mg) of sulfobromophthalein sodium $(C_{20}H_8Br_4Na_2O_{10}S_2)$ $= A/881 \times 200,000$

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.

Sulfur

イオウ

S: 32.07

Sulfur, when dried, contains not less than 99.5% of S.

Description Sulfur occurs as a light yellow to yellow powder. It is odorless and tasteless.

It is freely soluble in carbon disulfide, and practically insoluble in water, in ethanol (95) and in diethyl 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 develops.

(3) Boil 1 mg of sulfur with 2 mL of pyridine and 0.2 mL of sodium hydrogen carbonate TS: a blue color develops.

Purity (1) Clarity 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) Acidity or alkalinity—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 develops. Further add 1.0 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(3) Arsenic $\langle 1.11 \rangle$ —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 $\langle 2.41 \rangle$ Not more than 1.0% (1 g, in vacuum, not more than 0.67 kPa, silica gel, 4 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 on 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 dropwise until no more precipitate is formed, and heat on 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 (BaSO₄: 233.39). Perform a blank determination, and make any necessary correction.

Amount (mg) of S

= amount (mg) of barium sulfate (BaSO₄) \times 0.13739

Containers and storage Containers-Well-closed containers.

Sulfur and Camphor Lotion

イオウ・カンフルローション

Method of preparation

Sulfur				6	0 g	
<i>d</i> -Camphor or <i>dl</i> -Camphor					5 g	
Hydroxypropylcellulose					4 g	
Calcium Hydroxide					1 g	
Ethanol					4 n	nL
Water, Purified Water or Purified						
Water in Containers	а	suff	ficient	qua	ant	ity
	-			100	~	-

To make 1000 mL

Dissolve Hydroxypropylcellulose in 200 mL of Water, Purified Water or Purified Water in Containers. Add this solution in small portions to the triturate of Sulfur with the Ethanol solution of *d*-Camphor or *dl*-Camphor, and triturate again the mixture. Separately, dissolve Calcium Hydroxide in 500 mL of Water, Purified Water or Purified Water in Containers, stopper tightly, shake, and allow to stand. Add 300 mL of this supernatant liquid to the above mixture, then add Water, Purified Water or Purified Water in Containers to make 1000 mL, and shake thoroughly.

Description Sulfur and Camphor Lotion is a light yellow suspension.

A part of the components separates out on standing.

Identification (1) To 5 mL of well shaken Sulfur and Camphor Lotion add 25 mL of water, and centrifuge [use this supernatant liquid for test (3)]. To 0.02 g of the precipitate add 2 mL of pyridine and 0.2 mL of sodium hydrogen carbonate TS, and boil: a blue color develops (sulfur).

(2) To 10 mL of well shaken Sulfur and Comphor Lotion add 5 mL of diethyl ether, and mix. Separate the diethyl ether layer, and filter through a pledget of cotton. Wash the cotton with a small portion of diethyl ether, combine the washings with the filtrate, and distil cautiously on a water bath to remove the diethyl ether. Dissolve the residue in 1 mL of methanol, add 1 mL of 2,4-dinitrophenylhydrazine TS, and heat for about 2 minutes on a water bath. Cool, dilute with water to make about 5 mL, and allow to stand. Filter the produced precipitate through a glass filter (G4), and wash the residue on the filter with water until the last washing is colorless. Dissolve the residue in 10 mL of ethanol (95), add 5 mL of sodium hydroxide TS, and allow to stand for 2 minutes: a red color develops (*d*-camphor or *dl*-camphor).

(3) The supernatant liquid obtained in (1) responds to the Qualitative Tests $\langle 1.09 \rangle$ (2) and (3) for calcium salt.

Containers and storage Containers—Tight containers.

Sulfur, Salicylic Acid and Thianthol Ointment

イオウ・サリチル酸・チアントール軟膏

Method of preparation

Sulfur	100 g
Salicylic Acid, finely powdered	30 g
Thianthol	100 mL
Zinc Oxide, very finely powdered	100 g
Simple Ointment or a suitable	
ointment base	a sufficient quantity
	To make 1000 g

Prepare as directed under Ointments, with above ingredients.

Description Sulfur, Salicylic Acid and Thianthol Ointment is light yellow in color.

Identification (1) Stir well 0.5 g of Sulfur, Salicylic Acid and Thianthol Ointment with 10 mL of water while heating, cool, and filter. To 1 mL of the filtrate add 5 mL of iron (III) nitrate TS: a purple color is produced (salicylic acid).

(2) Shake 1 g of Sulfur, Salicylic Acid and Thianthol Ointment with 20 mL of diethyl ether, remove the supernatant liquid and floating materials. Wash the residue with 10 mL of diethyl ether, and remove the diethyl ether by suction. To the residue add 2 mL of pyridine and 0.2 mL of sodium hydrogen carbonate TS, and boil: a light blue to blue color is produced (sulfur).

(3) To 1 g of Sulfur, Salicylic Acid and Thianthol Ointment add 15 mL of ethanol (95), stir well while warming on a water bath, cool, and filter. Use the filtrate as the sample solution. Dissolve 0.01 g each of salicylic acid and thianthol in 5 mL of ethanol (95), and use these solutions as the standard solution (1) and standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 5 μ L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (45:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots of each component obtained from the sample solution and standard solutions (1) and (2) show the same Rfvalue. Spray iron (III) chloride TS upon the plate evenly: the spot from the standard solution (1) and that from the corresponding sample solution reveal a purple color.

Containers and storage Containers-Tight containers.

Sulindac



 $C_{20}H_{17}FO_3S: 356.41$ (1Z)-(5-Fluoro-2-methyl-1-{4-[(RS)-methylsulfinyl]benzylidene}-1H-inden-3-yl)acetic acid [38194-50-2]

Sulindac, when dried, contains not less than 99.0% and not more than 101.0% of $C_{20}H_{17}FO_3S$.

Description Sulindac occurs as a yellow, crystalline powder.

It is sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

A solution of Sulindac in methanol (1 in 100) shows no optical rotation.

Melting point: about 184°C (with decomposition).

Identification (1) Dissolve 15 mg of Sulindac in 1000 mL of a solution of hydrochloric acid in methanol (1 in 120). Determine the absorption spectrum of this solution as directed under the Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$ and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Sulindac as directed in the potassium bromide disc method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals $\langle 1.07 \rangle$ —Proceed with 2.0 g of Sulindac 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 $\langle 1.11 \rangle$ —Prepare the test solution with 1.0 g of Sulindac according to Method 3 and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.25 g of Sulindac in 10 mL of methanol and use this solution as the sample solution. Pipet 1 mL of the sample solution and add methanol to make exactly 100 mL. Pipet 5 mL, 4 mL and 2 mL of this solution, to each add methanol to make exactly 10 mL, and use these solutions as the standard solutions (1), (2) and (3). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 4 μ L each of the sample solution, standard solution (1), (2) and (3) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and acetic acid (100) (97:3) to a distance of about 17 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (1), and the total intensity of spots other than the principal spot from the sample solution is not more than 1.0% calculated

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on the basis of intensities of the spots from the standard solution (1), (2) and (3).

(4) Residual solvent Being specified separately.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, in vacuum not exceeding 0.7 kPa, 100°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 0.3 g of Sulindac, previously dried, dissolve in 50 mL of methanol and titrate $\langle 2.50 \rangle$ with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = $35.64 \text{ mg of } C_{20}H_{17}FO_3S$

Containers and storage Containers—Tight containers.

Sulpiride

スルピリド



C₁₅H₂₃N₃O₄S: 341.43 *N*-(1-Ethylpyrolidin-2-ylmethyl)-2-methoxy-5sulfamoylbenzamide [*15676-16-1*]

Sulpiride, when dried, contains not less than 98.5% and not more than 101.0% of $C_{15}H_{23}N_3O_4S$.

Description Sulpiride is a white, crystalline powder.

It is freely soluble in acetic acid (100) and in dilute acetic acid, sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

It is soluble in 0.05 mol/L sulfuric acid TS.

A solution of Sulpiride in methanol (1 in 100) shows no optical rotation.

Melting point: about 178°C (with decomposition).

Identification (1) Dissolve 0.1 g of Sulpiride in 0.05 mol/L sulfuric acid TS to make 100 mL. Dilute 5 mL of the solution with water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, using water as the blank, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Sulpiride as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Clarity 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 with the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>,

450 nm does not exceed 0.020.
(2) Heavy metals <1.07>—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 sample solution. Dilute 1 mL of the sample solution, accurately measured, with methanol to make exactly 100 mL. Dilute 2 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 these solutions as directed under Thinlayer Chromatography $\langle 2.03 \rangle$. Spot 20 μ L each of the sample solution and standard solution on a plate of silica gel with 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): the number of the spots other than the principal spot from the sample solution is not more than 2, and they have no more intense than the spot from the standard solution. When the plate is exposed to iodine vapor for 30 minutes, the number of the spots other than the principal spot from the sample solution is not more than 2, and they have no more intense than the spot from the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Dissolve about 0.4 g of Sulpiride, previously dried and accurately weighed, in 80 mL of acetic acid (100), and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS) until 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
=
$$34.14 \text{ mg}$$
 of $C_{15}H_{23}N_3O_4S$

Containers and storage Containers—Well-closed containers.

Sulpiride Capsules

スルピリドカプセル

Sulpiride Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of sulpiride ($C_{15}H_{23}N_3O_4S$: 341.43).

Method of preparation Prepare as directed under Capsules, with Sulpiride.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, using water as the blank: it exhibits a maximum between 289 nm and 293 nm.

Uniformity of dosage units $\langle 6.02 \rangle$ Perform the test according to the following method: it meets the requirement of the

JP XVI

Content uniformity test.

To 1 capsule of Sulpiride Capsules add 30 mL of 0.05 mol/L sulfuric acid TS, shake for 30 minutes, add 0.05 mol/L sulfuric acid TS to make exactly V mL so that each mL of the solution contains about 1 mg of sulpiride (C₁₅H₂₃N₃O₄S), and filter the solution. Discard the first 20 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of sulpiride (
$$C_{15}H_{23}N_3O_4S$$
)
= $M_S \times A_T/A_S \times V/50$

 $M_{\rm S}$: Amount (mg) of sulpiride for assay

Dissolution Being specified separately.

Assay Cut the capsule of not less than 20 Sulpiride Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of sulpiride ($C_{15}H_{23}N_3O_4S$), add 70 mL of 0.05 mol/L sulfuric acid TS, shake for 30 minutes, and add 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of sulpiride for assay, previously dried at 105°C for 3 hours, and dissolve in 0.05 m/L sulfuric acid TS to make exactly 50 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, $A_{\rm T}$ and $A_{\rm S}$, of the sample solution and standard solution at 291 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

> Amount (mg) of sulpiride ($C_{15}H_{23}N_3O_4S$) = $M_S \times A_T/A_S \times 2$

 $M_{\rm S}$: Amount (mg) of sulpiride for assay

Containers and storage Containers-Tight containers.

Sulpiride Tablets

スルピリド錠

Sulpiride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of sulpiride ($C_{15}H_{23}N_3O_4S$: 341.43).

Method of preparation Prepare as directed under Tablets, with Sulpiride.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, using water as the blank: it exhibits a maximum between 289 nm and 293 nm.

Uniformity of dosage units $\langle 6.02 \rangle$ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Sulpiride Tablets add 30 mL of 0.05 mol/L sulfuric acid TS, shake for 30 minutes, add 0.05 mol/L sulfuric acid TS to make exactly V mL so that each mL of the solution contains about 1 mg of sulpiride ($C_{15}H_{23}N_3O_4S$),

and filter the solution. Discard the first 20 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of sulpiride (C₁₅H₂₃N₃O₄S) = $M_{\rm S} \times A_{\rm T}/A_{\rm S} \times V/50$

 $M_{\rm S}$: Amount (mg) of sulpiride for assay

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate of a 50-mg tablet in 30 minutes is not less than 80%, that of a 100-mg tablet in 45 minutes is not less than 75%, and that of a 200-mg tablet in 45 minutes is not less than 70%.

Start the test with 1 tablet of Sulpiride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding $0.5 \,\mu\text{m}$. Discard the first 10 mL of the filtrate, pipet VmL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 56 μ g of sulpiride (C₁₅H₂₃N₃O₄S) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of sulpiride for assay, previously dried at 105°C for 3 hours, and dissolve in the dissolution medium to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 25 mL, and use this solution as the standard solution. Determine the absorbances, $A_{\rm T}$ and $A_{\rm S}$, at 291 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of sulpiride ($C_{15}H_{23}N_3O_4S$)

 $= M_{\rm S} \times A_{\rm T}/A_{\rm S} \times V'/V \times 1/C \times 180$

 $M_{\rm S}$: Amount (mg) of sulpiride for assay

C: Labeled amount (mg) of sulpiride (C₁₅H₂₃N₃O₄S) in 1 tablet

Assay Weigh accurately, and powder not less than 20 Sulpiride Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of sulpiride (C15H23N3O4S), add 70 mL of 0.05 mol/L sulfuric acid TS, shake for 30 minutes, and add 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of sulpiride for assay, previously dried at 105°C for 3 hours, and dissolve in 0.05 mL sulfuric acid TS to make exactly 50 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, $A_{\rm T}$ and $A_{\rm S}$, of the sample solution and standard solution at 291 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

Amount (mg) of sulpiride (C₁₅H₂₃N₃O₄S)
=
$$M_{\rm S} \times A_{\rm T}/A_{\rm S} \times 2$$

 $M_{\rm S}$: Amount (mg) of sulpiride for assay

Containers and storage Containers—Tight containers.

Sulpyrine Hydrate

スルピリン水和物



C₁₃H₁₆N₃NaO₄S.H₂O: 351.35 Monosodium [(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)(methyl)amino]methanesulfonate monohydrate [*5907-38-0*]

Sulpyrine Hydrate contains not less than 98.5% of sulpyrine ($C_{13}H_{16}N_3NaO_4S$: 333.34), calculated on the dried basis.

Description Sulpyrine Hydrate occurs as white to light yellow crystals or crystalline powder. It is odorless, and has a bitter taste.

It is very soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

It is colored by light.

Identification (1) Add 2 drops of dilute sulfuric acid and 1 mL of chlorinated lime TS to 3 mL of a solution of Sulpyrine Hydrate (1 in 15): a deep blue color develops at first, but the color immediately turns red, then gradually changes to yellow.

(2) Boil 5 mL of a solution of Sulpyrine Hydrate (1 in 25) with 3 mL of dilute hydrochloric acid: the odor of sulfur dioxide is perceptible at first, and on further boiling, the odor of formaldehyde is perceptible.

(3) A solution of Sulpyrine Hydrate (1 in 10) responds to the Qualitative Tests $\langle 1.09 \rangle$ for sodium salt.

Purity (1) Clarity of solution, and acidity or alkalinity—Dissolve 1.0 g of Sulpyrine Hydrate in 10 mL of water: the solution is clear and neutral.

(2) Sulfate $\langle 1.14 \rangle$ —Dissolve 0.20 g of Sulpyrine Hydrate in 0.05 mol/L hydrochloric acid VS 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 and 0.05 mol/L hydrochloric acid VS to make 50 mL (not more than 0.120%).

(3) Heavy metals $\langle 1.07 \rangle$ —Proceed with 1.0 g of Sulpyrine 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) Merbuline—Transfer 0.10 g of Sulpyrine Hydrate with 2 mL of water and 1 mL of dilute sulfuric acid into a flask, cover with a funnel, and boil gently for 15 minutes. Cool, add 2 mL of a solution of sodium acetate trihydrate (1 in 2) and water to make 5 mL, shake this solution with 5 mL of benzaldehyde-saturated solution, and allow to stand for 5 minutes: the solution is clear.

(5) Chloroform-soluble substances—Mix, by frequent shaking, 1.0 g of Sulpyrine Hydrate and 10 mL of chloroform for 30 minutes. Collect the precipitate, wash with two 5-mL portions of chloroform, combine the washings

with the filtrate, and evaporate on a water bath to dryness. Dry the residue at 105 °C for 4 hours: the mass of the residue is not more than 5.0 mg.

Loss on drying $\langle 2.41 \rangle$ Not more than 6.0% (1 g, 105°C, 4 hours).

Assay Weigh accurately about 0.25 g of Sulpyrine Hydrate, dissolve in 100 mL of diluted hydrochloric acid (1 in 20), previously cooled below 10°C. Titrate $\langle 2.50 \rangle$ immediately with 0.05 mol/L iodine VS while keeping the temperature between 5°C and 10°C, until the color of the solution remains blue upon shaking vigorously for 1 minute after the addition of 0.05 mol/L iodine VS (indicator: 1 mL of starch TS).

Each mL of 0.05 mol/L iodine VS = $16.67 \text{ mg of } C_{13}H_{16}N_3NaO_4S$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Sulpyrine Injection

スルピリン注射液

Sulpyrine Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of sulpyrine hydrate ($C_{13}H_{16}N_3NaO_4S.H_2O:$ 351.35).

Method of preparation Prepare as directed under Injections, with Sulpyrine Hydrate.

Description Sulpyrine Injection is a clear, colorless or pale yellow liquid.

pH: 5.0 – 8.5

Identification (1) To a volume of Sulpyrine Injection, equivalent to 0.2 g of Sulpyrine Hydrate according to the labeled amount, add water to make 3 mL, then add 2 drops of dilute sulfuric acid and 1 mL of chlorinated lime TS: a deep blue color develops at first, and the color immediately turns red and gradually changes to yellow.

(2) To a volume of Sulpyrine Injection, equivalent to 0.2 g of Sulpyrine Hydrate according to the labeled amount, add water to make 5 mL, and boil this solution with 3 mL of dilute hydrochloric acid: the odor of sulfur dioxide is perceptible at first, and on further boiling the odor of formalde-hyde is perceptible.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter $\langle 6.06 \rangle$ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Pipet 2 mL of Sulpyrine Injection, dilute with water to exactly 100 mL. Measure exactly a volume (V mL) of this solution, equivalent to about 50 mg of sulpyrine hydrate ($C_{13}H_{16}N_3NaO_4S.H_2O$), and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to exactly 100 mL, and use this solution as the sample solution. Weigh accurately about 50 mg of sulpyrine for assay (previously determine the loss on drying $\langle 2.41 \rangle$ in the same conditions as Sulpyrine Hydrate), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to exactly 100 mL, and use this solution as the standard solution. Pipet 2 mL each of the sample solution and standard solution into separate 25-mL volumetric flasks, add 5 mL of ethanol (95), 2 mL of a solution of 4-dimethylaminocinnamaldehyde in ethanol (95) (1 in 250) and 2 mL of acetic acid (100) to each of these solutions, shake well, allow to stand for 15 minutes, and add water to exactly 25 mL. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with 2 mL of water in the same manner as the blank. Determine the absorbances, $A_{\rm T}$ and $A_{\rm S}$, of the subsequent solutions of the sample solution and the standard solution at 510 nm.

Amount (mg) of sulpyrine hydrate ($C_{13}H_{16}N_3NaO_4S.H_2O$) in 1 mL of Sulpyrine Injection

 $= M_{\rm S} \times A_{\rm T}/A_{\rm S} \times 50/V \times 1.054$

 $M_{\rm S}$: Amount (mg) of sulpyrine for assay, calculated on the dried basis

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage-Light-resistant, and under nitrogen atmosphere.

Sultamicillin Tosilate Hydrate

スルタミシリントシル酸塩水和物



 $C_{25}H_{30}N_4O_9S_2.C_7H_8O_3S.2H_2O: 802.89 \\ (2S,5R)-(3,3-Dimethyl-4,4,7-trioxo-4-thia-1-azabicyclo[3.2.0]hept-2-ylcarbonyloxy)methyl \\ (2S,5R,6R)-6-[(2R)-2-amino-2-phenylacetylamino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate mono-4-toluenesulfonate dihydrate [83105-70-8, anhydride]$

Sultamicillin Tosilate Hydrate contains not less than 698 μ g (potency) and not more than 800 μ g (potency) per mg, calculated on the anhydrous basis and corrected by the amount of residual solvent. The potency of Sultamicillin Tosilate Hydrate is expressed as mass (potency) of sultamicillin (C₂₅H₃₀N₄O₉S₂: 594.66).

Description Sultamicillin Tosilate Hydrate occurs as a white to yellowish white crystalline powder.

It is freely soluble in acetonitrile, in methanol and in ethanol (99.5), and very slightly soluble in water.

Identification Determine the infrared absorption spectrum of Sultamicillin Tosilate Hydrate as directed in the paste

method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Sultamicillin Tosilate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_{D}^{20}$: $+173 - +187^{\circ}$ (0.5 g calculated on the anhydrous bases, a mixture of water and acetonitrile (3:2), 25 mL, 100 mm).

Purity (1) Heavy metals $\langle 1.07 \rangle$ —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 Standard Lead Solution (not more than 20 ppm).

(2) Arsenic $\langle 1.11 \rangle$ —Prepare the test solution 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 sample solution. Separately, weigh accurately an amount of Ampicillin RS, equivalent to about 20 mg (potency), 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 exactly 25 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the area of the peak of ampicillin by the automatic integration method: the peak area from the sample 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 to pH 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 μ L 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 $25 \,\mu\text{L}$ 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 sample solution. Separately, weigh accurately an amount of Sulbactam RS, equivalent to about 20 mg (potency), 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 exactly 25 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following con-

ditions, and determine the area of the peak of sulbactam by the automatic integration method: the peak area from the sample 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 acids—Weigh accurately about 25 mg of Sultamicillin Tosilate Hydrate, dissolve in 1 mL of acetonitrile, and add 25 mL of 0.02 mol/L phosphate buffer solution, pH 3.0, in a 100-mL flask with stopper. Add exactly 5 mL of 0.005 mol/L iodine VS, and allow to stand the stoppered flask for 5 minutes. Titrate $\langle 2.50 \rangle$ with 0.005 mol/L sodium thiosulfate VS (indicator: 1.0 mL of starch TS). Perform a blank determination in the same manner, and make any necessary correction. Calculate the amount of penicilloic acid (C₂₅H₃₄N₄O₁₁S₂: 630.70) by using the following equation: it 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 $\langle 2.46 \rangle$ —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 sample solution. Separately, weigh accurately about 1 g of ethyl acetate, and mix with water to make exactly 200 mL. Pipet 2 mL of this solution, add 10 mL of methanol and water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly $5 \,\mu$ L each of the sample solution and standard solution as directed under Gas Chromatography $\langle 2.02 \rangle$ according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of ethyl acetate of these solutions. Calculate the amount of ethyl acetate by the following equation: not more than 2.0%.

Amount (%) of ethyl acetate
=
$$M_{\rm S}/M_{\rm T} \times A_{\rm T}/A_{\rm S} \times 1/5$$

 $M_{\rm S}$: Amount (mg) of ethyl acetate $M_{\rm T}$: Amount (mg) of the sample

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A column 3 mm in inside diameter and 1 m in length, packed with porous styrene-divinylbenzene copolymer for gas chromatography (average pore diameter: 0.0085 μ m, 300 – 400 m²/g) (150 to 180 μ m 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 $5 \,\mu L$ of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ethyl acetate are not less than 500 steps and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with $5 \,\mu$ L 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 $\langle 2.48 \rangle$ 4.0 – 6.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.2% (1 g).

Assay Perform the procedure rapidly. Weigh accurately an amount of Sultamicillin Tosilate Hydrate and Sultamicillin Tosilate RS, equivalent to about 50 mg (potency), dissolve each in the mobile phase to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, add the mobile phase to make 25 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μ L each of these solutions as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of sultamicillin to that of the internal standard of each solution.

Amount [μ g (potency)] of sultamicillin (C₂₅H₃₀N₄O₉S₂) = $M_{\rm S} \times Q_{\rm T}/Q_{\rm S} \times 1000$

M_S: Amount [mg (potency)] of Sultamicillin Tosilate RS

Internal standard solution—A solution of isopropyl-4aminobenzoate in the mobile phase (1 in 2500).

Operating conditions—

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

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about $35^{\circ}C$.

Mobile phase: Dissolve 3.12 g of sodium dihydrogenphosphate in about 750 mL of water, adjust to pH 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 μ L 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 $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sultamicillin is not more than 2.0%.

Containers and storage Containers—Tight containers.

Official Monographs / Suxamethonium Chloride Hydrate 1447

Sultiame

スルチアム



 $C_{10}H_{14}N_2O_4S_2$: 290.36 4-(3,4,5,6-Tetrahydro-2*H*-1,2-thiazin-2-yl)benzenesulfonamide *S*,*S*-dioxide [61-56-3]

Sultiame, when dried, contains not less than 98.5% of $C_{10}H_{14}N_2O_4S_2$.

Description Sultiame occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is very soluble in N, N-dimethylformamide, freely soluble in n-butylamine, slightly soluble in methanol and in ethanol (95), very slightly soluble in water, and practically insoluble in diethyl ether.

It dissolves in sodium hydroxide TS.

Identification (1) Dissolve 0.02 g of Sultiame in 5 mL of water and 1 mL of *n*-butylamine, add 2 to 3 drops of copper (II) sulfate TS, and shake well. To this solution add 5 mL of chloroform, shake, and allow to stand: a green color develops in the chloroform layer.

(2) Mix 0.1 g of Sultiame with 0.5 g of sodium carbonate decahydrate, and melt carefully: the gas evolved changes moistened red litmus paper to blue. After cooling, crush the fused substance with a glass rod, stir with 10 mL of water, and filter. To 4 mL of the filtrate add 2 drops of hydrogen peroxide (30), 5 mL of diluted hydrochloric acid (1 in 5) and 2 to 3 drops of barium chloride TS: a white precipitate is formed.

(3) Determine the absorption spectrum of a solution of Sultiame in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point <2.60> 185 – 188°C

Purity (1) Chloride $\langle 1.03 \rangle$ —Dissolve 1.0 g of Sultiame in 20 mL of sodium hydroxide TS by warming, cool, and add 2 mL of acetic acid (100) and water to make 100 mL. After shaking, filter, and discard the first 10 mL of the filtrate. To 40 mL of the subsequent filtrate 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 as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 8 mL of sodium hydroxide TS, 0.8 mL of acetic acid (100), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.022%).

(2) Sulfate $\langle 1.14 \rangle$ —Dissolve 1.0 g of Sultiame in 20 mL of sodium hydroxide TS by warming, cool, and add 8 mL of dilute hydrochloric acid and water to make 100 mL. After shaking, filter, and discard the first 10 mL of the filtrate. To 40 mL of the subsequent 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 as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS add 8 mL of sodium hydroxide TS, 4.2 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(3) Heavy metals $\langle 1.07 \rangle$ —Proceed with 2.0 g of Sultiame 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 $\langle 1.11 \rangle$ —Prepare the test solution with 1.0 g of Sultiame according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Sultiame in methanol to make exactly 20 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of sulfanilamide in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add methanol 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 $\langle 2.03 \rangle$. Spot 20 μ L each of the sample solution and 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) (30:8:1) 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 from the sample solution are not more intense than the spot from the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.8 g of Sultiame, previously dried, dissolve in 70 mL of N,N-dimethylformamide, and titrate $\langle 2.50 \rangle$ with 0.2 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.2 mol/L tetramethylammonium hydroxide VS = $58.07 \text{ mg of } C_{10}H_{14}N_2O_4S_2$

Containers and storage Containers—Well-closed containers.

Suxamethonium Chloride Hydrate

スキサメトニウム塩化物水和物



 $C_{14}H_{30}Cl_2N_2O_4.2H_2O:$ 397.34 2,2'-Succinyldioxybis(*N*,*N*,*N*-trimethylethylaminium) dichloride dihydrate [6101-15-1]

Suxamethonium Chloride Hydrate contains not less than 98.0% of suxamethonium chloride $(C_{14}H_{30}Cl_2N_2O_4: 361.31)$, calculated on the anhydrous basis.

Description Suxamethonium Chloride Hydrate occurs as a

white, crystalline powder.

It is freely soluble in water, in methanol and in acetic acid (100), slightly soluble in ethanol (95), very slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Identification (1) Determine the infrared absorption spectrum of Suxamethonium Chloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Suxamethonium Chloride Hydrate (1 in 20) responds to the Qualitative Tests <1.09> for chloride.

pH $\langle 2.54 \rangle$ The pH of a solution of Suxamethonium Chloride Hydrate (1 in 100) is between 4.0 and 5.0.

Melting point <2.60> 159 – 164°C (hydrate form).

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 sample solution. Pipet 1 mL of the sample 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 <2.03>. Spot 1 μ L each of the sample solution and 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, n-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: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water $\langle 2.48 \rangle$ 8.0 - 10.0% (0.4 g, volumetric titration, direct titration).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Suxamethonium Chloride Hydrate, dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration).

Each mL of 0.1 mol/L perchloric acid VS = $18.07 \text{ mg of } C_{14}H_{30}Cl_2N_2O_4$

Containers and storage Containers—Tight containers.

Suxamethonium Chloride for Injection

注射用スキサメトニウム塩化物

Suxamethonium Chloride for Injection is a preparation for injection which is dissolved before use.

It 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 for

Injection should be 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 for Injection occurs as a white, crystalline powder or mass.

Identification Take an amount of Suxamethonium Chloride for Injection, equivalent to 0.05 g of Suxamethonium Chloride Hydrate according to the labeled amount, dissolve in water to make 10 mL, and use this solution as the sample solution. Separately, dissolve 0.05 g of suxamethonium chloride for thin-layer chromatography 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 $\langle 2.03 \rangle$. Spot 1 μ L each of the sample solution and 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 evenly hydrogen hexachloroplatinate (IV)-potassium iodide TS on the plate: the spots obtained from the sample solution and standard solution are blue-purple in color and have similar Rf value.

pH $\langle 2.54 \rangle$ The pH of a solution of Suxamethonium Chloride for Injection (1 in 100) is between 4.0 and 5.0.

Purity Related substances—Take 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.

Bacterial endotoxins <4.01> Less than 1.5 EU/mg.

Uniformity of dosage units $\langle 6.02 \rangle$ It meets the requirement of the Mass variation test.

Foreign insoluble matter $\langle 6.06 \rangle$ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: 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 = $18.07 \text{ mg of } C_{14}H_{30}Cl_2N_2O_4$

Containers and storage Containers-Hermetic containers.

Suxamethonium Chloride Injection

スキサメトニウム塩化物注射液

Suxamethonium Chloride Injection is an aqueous solution for injection.

It 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 should be 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, colorless liquid.

Identification Take a volume of Suxamethonium Chloride Injection, equivalent to 0.05 g of Suxamethonium Chloride Hydrate according to the labeled amount, add water to make 10 mL, and use this solution as the sample solution. Separately, dissolve 0.05 g of suxamethonium chloride for thinlater chromatography 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 <2.03>. Spot 1 μ L each of the sample solution and 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 evenly hydrogen hexachloroplatinate (IV)-potassium iodide TS on the plate: the spots obtained from the sample solution and the standard solution are blue-purple in color and have similar Rf value.

pH <2.54> 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 200 mg of Suxamethonium Chloride ($C_{14}H_{30}Cl_2N_2O_4$) taken.

Bacterial endotoxins <4.01> Less than 2.0 EU/mg.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Transfer to a separator an accurately measured volume of Suxamethonium Chloride Injection, equivalent to about 0.2 g of suxamethonium chloride $(C_{14}H_{30}Cl_2N_2O_4)$, add 30 mL of freshly boiled and cooled water, and wash the solution with five 20-mL portions of diethyl ether. Combine the diethyl ether washings, and extract the combined diethyl ether layer with two 10-mL portions of freshly boiled and cooled water. Wash the combined water extracts with two 10-mL portions of diethyl ether solution and

the water extracts, add 2 drops of bromothymol 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 $\langle 2.50 \rangle$ 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 bromothymol blue TS, neutralize the solution with 0.1 mol/L sodium hydroxide VS, and perform a blank determination.

Each mL of 0.1 mol/L sodium hydroxide VS = 18.07 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.

Tacrolimus Hydrate

タクロリムス水和物



C44H69NO12.H2O: 822.03

(3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-5,19-Dihydroxy-3-{(1*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylethenyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-8-(prop-2-en-1-yl)-15,19-epoxy-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26ahexadecahydro-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone monohydrate [*109581-93-3*]

Tacrolimus Hydrate contains not less than 98.0% and not more than 102.0% of tacrolimus (C₄₄H₆₉NO₁₂: 804.02), calculated on the anhydrous basis.

Description Tacrolimus Hydrate occurs as a white crystal or crystalline powder.

It is very soluble in methanol and in ethanol (99.5), freely soluble in N,N-dimethylformamide and in ethanol (95), and practically insoluble in water.

Identification (1) Dissolve 5 mg of Tacrolimus Hydrate in 1 mL of ethanol (95), add 1 mL of 1,3-dinitrobenzene TS and 1 mL of sodium hydroxide TS, and shake: a red-purple color develops.

(2) Determine the infrared absorption spectrum of Tacrolimus Hydrate as directed in the paste method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Tacrolimus RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_D^{25}$: $-112 - -117^\circ$ (0.2 g calculated on the anhydrous basis, *N*,*N*-dimethylformamide, 20 mL, 100 mm).

Purity (1) Heavy metals $\langle 1.07 \rangle$ —Proceed with 2.0 g of Tacrolimus 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) Related substances—Being specified separately.
- (3) Residual solvent—Being specified separately.

Water $\langle 2.48 \rangle$ 1.9 – 2.5% (0.5 g, volumetric titration, direct titration).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Isomer Being specified separately.

Assay Weigh accurately about 25 mg each of Tacrolimus Hydrate and Tacrolimus RS (separately determine the water $\langle 2.48 \rangle$ in the same manner as Tacrolimus Hydrate) and dissolve each in 15 mL of ethanol (99.5), to each add exactly 10 mL of the internal standard solution, add 25 mL of water, allow to stand for 6 hours, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of tacrolimus to that of the internal standard.

Amount (mg) of tacrolimus ($C_{44}H_{69}NO_{12}$) = $M_S \times Q_T/Q_S$

 $M_{\rm S}$: Amount (mg) of Tacrolimus RS, calculated on the anhydrous basis

Internal standard solution—A solution of heptyl parahydroxybenzoate in ethanol (99.5) (3 in 4000).

Operating conditions—

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

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

Column temperature: A constant temperature of about 50° C.

Mobile phase: A mixture of water, 2-propanol for liquid chromatography and tetrahydrofuran for liquid chromatography (5:2:2).

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

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, tacrolimus 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 $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of tacrolimus to that of internal standard is not more than 1.0%.

Containers and storage Containers-Well-closed containers.

Talampicillin Hydrochloride

Ampicillinphthalidyl Hydrochloride

タランピシリン塩酸塩



 $C_{24}H_{23}N_3O_6S.HCl: 517.98$ 3-Oxo-1,3-dihydroisobenzofuran-1-yl (2*S*,5*R*,6*R*)-6-[(2*R*)-2-amino-2-phenylacetylamino]-3,3-dimethyl-7-oxo-4thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrochloride [47747-56-8]

Talampicillin Hydrochloride is the hydrochloride of ampicillin phthalidyl ester.

It contains not less than $600 \,\mu g$ (potency) and not more than $700 \,\mu g$ (potency) per mg, calculated on the anhydrous basis. The potency of Talampicillin Hydrochloride is expressed as mass (potency) of ampicillin (C₁₆H₁₉N₃O₄S: 349.40).

Description Talampicillin Hydrochloride occurs as a white to light yellowish white powder.

It is very soluble in methanol, and freely soluble in water and in ethanol (99.5).

Identification (1) To 1 mL of a solution of Talampicillin Hydrochloride (1 in 30) add 1 mL of sodium hydroxide TS, mix, allow to stand for 5 minutes, and add 2 mL of dilute sulfuric acid and 2 to 3 drops of 2,4-dinitrophenylhydrazine TS: an orange-yellow precipitate is formed.

(2) Determine the infrared absorption spectrum of Talampicillin Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Talampicillin Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 10 mL of a solution of Talampicillin Hydrochloride (1 in 300) add 1 mL of dilute nitric acid, and add silver nitrate TS: a white precipitate is formed.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_D^{20}$: $+151 - +171^\circ$ (0.2 g calculated on the anhydrous basis, ethanol (99.5), 20 mL, 100 mm).

Purity (1) Heavy metals $\langle 1.07 \rangle$ —Proceed with 1.0 g of Talampicillin 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) Arsenic $\langle 1.11 \rangle$ —Prepare the test solution with 1.0 g of Talampicillin Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Talampicillin Hydrochloride in ethanol (99.5) to make exactly 10 mL, and use this solution as the sample solution. Pipet 1 mL, 2 mL

and 3 mL of the sample solution, add ethanol (99.5) to each to make exactly 100 mL, and use these solutions as the standard solution (1), the standard solution (2) and the standard solution (3), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot $10 \,\mu L$ each of the sample 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 tetrahydrofuran, ethyl acetate, water and ethanol (95) (4:4:2:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in ethanol (99.5) (1 in 500) on the plate, and heat at 110°C for 5 minutes: the spots other than the principal spot from the sample solution is not more intense than the spot from the standard solution (3), and the total of the amount of each spot other than the principal spot from the sample solution, which is calculated by the comparison with the spots obtained from the standard solutions (1), (2) and (3), is not more than 5%.

(4) 2-Formylbenzoic acid—Dissolve 50 mg of Talampicillin Hydrochloride in ethanol (99.5) to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of 2-formylbenzoic acid in ethanol (99.5) to make exactly 100 mL. Pipet 5 mL of this solution, add ethanol (99.5) 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 <2.03>. Spot $10 \,\mu\text{L}$ each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of chloroform and acetic acid (100) (4:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly a solution of 2,4-dinitrophenylhydrazine in diluted sulfuric acid (6 in 25) (1 in 500): the spot of 2formylbenzoic acid obtained from the sample solution is not more intense than that obtained from the standard solution.

Water $\langle 2.48 \rangle$ Not more than 3.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Talampicillin Hydrochloride and Talampicillin Hydrochloride RS, equivalent to about 20 mg (potency), dissolve in water to make exactly 20 mL each, and use these solutions as the sample solution and the standard solution. The standard solution should be prepared before use. Pipet 2 mL each of the sample solution and the standard solution in separate 100-mL glassstoppered flasks, add 2.0 mL of sodium hydroxide TS, and allow them to stand for exactly 15 minutes. Add 2.0 mL of diluted hydrochloric acid (1 in 10) and exactly 10 mL of 0.005 mol/L iodine VS, allow them to stand for exactly 15 minutes, and titrate <2.50> with 0.01 mol/L sodium thiosulfate VS until the color of the solution is disappeared. If necessary, add 0.2 to 0.5 mL of starch TS. Separately, pipet 2 mL each of the sample solution and the standard solution in separate 100-mL glass-stoppered flasks, add exactly 10 mL of 0.005 mol/L iodine VS, titrate <2.50> with 0.01 mol/L sodium thiosulfate VS until the color of the solution is disappeared, and make any necessary correction. For this titration, add 0.2 to 0.5 mL of starch TS, if necessary. Calculate the amount (mL) of 0.005 mol/L iodine VS, $V_{\rm T}$ and $V_{\rm S}$, consumed by the sample solution and the standard solution, respectively.

Amount [μ g (potency)] of ampicillin (C₁₆H₁₉N₃O₄S) = $M_{\rm S} \times V_{\rm T}/V_{\rm S} \times 1000$ $M_{\rm S}$: Amount [mg (potency)] of Talampicillin Hydrochloride RS

Containers and storage Containers—Tight containers.

Talc

タルク

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (\bullet).

Talc is a powdered, selected, natural, hydrated magnesium silicate. Pure talc is $Mg_3Si_4O_{10}(OH)_2$: 379.27. It may contain related mineral substances consisting chiefly of chlorite (hydrous magnesium aluminum silicate), magnesite (magnesium carbonate), calcite (calcium carbonate) and dolomite (calcium magnesium carbonate).

It contains no asbestos.

It contains not less than 17.0% and not more than 19.5% of magnesium (Mg: 24.31).

•**Description** Talc occurs as a white to grayish white, fine, crystalline powder.

It is unctuous, and adheres readily to the skin.

It is practically insoluble in water and in ethanol (99.5).

Identification Determine the infrared absorption spectrum of Talc as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$: it exhibits absorption at the wave numbers of about 3680 cm⁻¹, 1018 cm⁻¹ and 669 cm⁻¹.

Purity (1) Acidity or alkalinity—To 2.5 g of Talc add 50 mL of freshly boiled and cooled water, and heat under a reflux condenser. Filter the liquid by suction, add 0.1 mL of bromothymol blue-sodium hydroxide-ethanol TS to 10 mL of the filtrate, and add 0.01 mol/L hydrochloric acid VS until the color of the solution changes: the necessary volume of the VS is not more than 0.4 mL. Separately, to 10 mL of the filtrate add 0.1 mL of phenolphthalein TS, and add 0.01 mol/L sodium hydroxide VS until the color of the solution changes to light red: the necessary volume of the VS is not more than 0.3 mL.

◆(2) Acid-soluble substances—Weigh accurately about 1 g of Talc, heat with 20 mL of dilute hydrochloric acid at 50°C for 15 minutes with stirring. Cool, add water to make exactly 50 mL, and filter. Centrifuge, if necessary, until the filtrate becomes clear. To 25 mL of the filtrate add 1 mL of dilute sulfuric acid, evaporate to dryness, and ignite to constant mass at 800 ± 25°C: the amount of the residue is not more than 2.0%.

(3) Water-soluble substances—To 10.0 g of Talc add 50 mL of water, weigh the mass, and boil for 30 minutes, supplying water lost by evaporation. Cool, add water to restore the original mass, and filter. Centrifuge, if necessary, until the filtrate becomes clear. Evaporate 20 mL of the filtrate to dryness, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 4.0 mg. ◆

(4) Iron—Weigh accurately about 10 g of Talc, add 50 mL of 0.5 mol/L hydrochloric acid TS gently while stirring, and heat under a reflux condenser on a water bath for 30

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minutes. After cooling, transfer the content to a beaker, and allow to settle the insoluble matter. Filter the supernatant liquid through a filter paper for quantitative analysis (No. 5B), leaving the precipitate in the beaker as much as possible, wash the remaining precipitate in the beaker with three 10-mL portions of hot water, and also wash the filter paper with 15 mL of hot water, and combine the washings and the filtrate. After cooling, add water to make exactly 100 mL, and use this solution as the sample stock solution. Pipet 2.5 mL of the stock solution, add 50 mL of 0.5 mol/L hydrochloric acid TS, then add water to make exactly 100 mL, and use this solution as the sample solution. Separately, to 50 mL each of 0.5 mol/L hydrochloric acid TS add exactly 2 mL, 2.5 mL, 3 mL and 4 mL of Standard Iron Solution for Atomic Absorption Spectrophotometry, add water to make them exactly 100 mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions, and calculate the amount of iron from the calibration curve prepared from the absorbances of the standard solutions: not more than 0.25%.

Gas: Combustible gas-Acetylene.

Supporting gas—Air.

Lamp: Iron hollow-cathode lamp.

Wavelength: 248.3 nm.

(5) Aluminum—Pipet 5 mL of the sample stock solution obtained in the Assay, add 10 mL of cesium chloride TS and 10 mL of hydrochloric acid, then add water to make exactly 100 mL, and use this solution as the sample solution. Separately, to 10 mL of hydrochloric acid and 10 mL of cesium chloride TS add exactly 5 mL, 10 mL, 15 mL and 20 mL of Standard Aluminum Solution for Atomic Absorption Spectrophotometry, add water to make them exactly 100 mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry $\langle 2.23 \rangle$ according to the following conditions, and calculate the amount of aluminum from the calibration curve prepared from the absorbances of the standard solutions: not more than 2.0%.

Gas: Combustible gas-Acetylene.

Supporting gas—Nitrous oxide.

Lamp: Aluminum hollow-cathode lamp.

Wavelength: 309.3 nm.

(6) Lead—Use the sample stock solution obtained in (4) as the sample solution. Separately, to 50 mL of 0.5 mol/L hydrochloric acid TS add exactly 5 mL, 7.5 mL, 10 mL and 12.5 mL of Standard Lead Solution, add water to make them exactly 100 mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry $\langle 2.23 \rangle$ according to the following conditions, and calculate the amount of lead from the calibration curve prepared from the absorbances of the standard solutions: not more than 10 ppm.

Gas: Combustible gas—Acetylene.

Supporting gas—Air.

Lamp: Lead hollow-cathode lamp.

Wavelength: 217.0 nm.

(7) Calcium—Pipet 5 mL of the sample stock solution obtained in the Assay, add 10 mL of hydrochloric acid and 10 mL of lanthanum chloride TS, then add water to make

exactly 100 mL, and use this solution as the sample solution. Separately, to 10 mL of hydrochloric acid and 10 mL of lanthanum chloride TS add exactly 1 mL, 2 mL, 3 mL and 5 mL of Standard Calcium Solution, add water to make them exactly 100 mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry $\langle 2.23 \rangle$ according to the following conditions, and calculate the amount of calcium from the calibration curve prepared from the absorbances of the standard solutions: not more than 0.9%.

Gas: Combustible gas—Acetylene.

Supporting gas—Nitrous oxide.

Lamp: Calcium hollow-cathode lamp.

Wavelength: 422.7 nm.

•(8) Arsenic $\langle 1.11 \rangle$ —To 0.5 g of Talc add 5 mL of dilute sulfuric acid, and heat gently to boiling with shaking. Cool immediately, filter, and wash the residue with 5 mL of dilute sulfuric acid, then with 10 mL of water. Combine the filtrate and the washings, evaporate to 5 mL on a water bath, and perform the test with this solution as the test solution (not more than 4 ppm).

Loss on ignition $\langle 2.43 \rangle$ Not more than 7.0% (1 g, 1050 – 1100°C, constant mass).

Assay Weigh accurately about 0.5 g of Talc in a polytetrafluoroethylene dish, add 5 mL of hydrochloric acid, 5 mL of nitric acid and 5 mL of perchloric acid, then add 35 mL of hydrofluoric acid while mixing gently, and evaporate to dryness on a hot plate by heating gradually. Add 5 mL of hydrochloric acid to the residue, cover the dish with a watch glass, and heat to boil. After cooling, transfer the content to a volumetric flask while washing the watch glass and dish with water, further wash the dish with water, transfer the washings to the flask, then add water to make exactly 50 mL, and use this solution as the sample stock solution. Pipet 0.5 mL of the sample stock solution, and add water to make exactly 100 mL. Pipet 4 mL of this solution, add 10 mL of hydrochloric acid and 10 mL of lanthanum chloride TS, then add water to make exactly 100 mL, and use this solution as the sample solution. Separately, to 10 mL of hydrochloric acid and 10 mL of lanthanum chloride TS add exactly 2.5 mL, 3 mL, 4 mL and 5 mL of Standard Magnesium Solution for Atomic Absorption Spectrophotometry, add water to make them exactly 100 mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions, and calculate the amount of magnesium from the calibration curve prepared from the absorbances of the standard solutions.

Gas: Combustible gas—Acetylene. Supporting gas—Air. Lamp: Magnesium hollow-cathode lamp. Wavelength: 285.2 nm.

◆Containers and storage Containers—Well-closed containers.

Tamoxifen Citrate

タモキシフェンクエン酸塩



 $C_{26}H_{29}NO.C_6H_8O_7$: 563.64 2-{4-[(1Z)-1,2-Diphenylbut-1-en-1-yl]phenoxy}-*N*,*N*-dimethylethylamine monocitrate [54965-24-1]

Tamoxifen Citrate, when dried, contains not less than 99.0% and not more than 101.0% of $C_{26}H_{29}NO$. $C_6H_8O_7$.

Description Tamoxifen Citrate occurs as a white crystalline powder.

It is freely soluble in acetic acid (100), sparingly soluble in methanol, and slightly soluble in water and in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Tamoxifen Citrate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tamoxifen Citrate as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Tamoxifen Citrate (1 in 100) responds to the Qualitative Tests <1.09> (1) for citrate.

Purity (1) Heavy metals $\langle 1.07 \rangle$ —Proceed with 2.0 g of Tamoxifen 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).

(2) Related substances—Conduct this procedure rapidly, using light-resistant vessels. Dissolve 15 mg of Tamoxifen Citrate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak other than tamoxifen obtained from the sample 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 the peak of tamoxifen from the sample 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).

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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 μ m in particle diameter).

Column temperature: 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 to pH 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.

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

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of tamoxifen obtained with 10 μ L of this solution is equivalent to 8 to 12% of that with 10 μ L of the standard solution.

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of tamoxifen are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ 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%.

(3) Residual solvent—Being specified separately.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 1 g of Tamoxifen Citrate, previously dried, dissolve in 150 mL of acetic acid (100), and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform the blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 56.36 mg of $C_{26}H_{29}NO.C_6H_8O_7$

Containers and storage Containers-Well-closed containers.

Storage—Light-resistant.

Tamsulosin Hydrochloride

タムスロシン塩酸塩



C₂₀H₂₈N₂O₅S.HCl: 444.97

5-{(2*R*)-2-[2-(2-Ethoxyphenoxy)ethylamino]propyl}-2-methoxybenzenesulfonamide monohydrochloride [*106463-17-6*]

Tamsulosin Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of $C_{20}H_{28}N_2O_5S$.HCl.

Description Tamsulosin Hydrochloride occurs as white crystals.

It is freely soluble in formic acid, sparingly 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 spectrum of a solution of Tamsulosin Hydrochloride (3 in 160,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tamsulosin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: 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 at room temperature for 30 minutes, and filter: the filtrate responds to the Qualitative Tests $\langle 1.09 \rangle$ for chloride.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_D^{20}$: $-17.5 - -20.5^\circ$ (after drying, 0.15 g, water, warming, after cooling, 20 mL, 100 mm).

Purity (1) Heavy metals $\langle 1.07 \rangle$ —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 sample solution. Pipet 2 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 2.5 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 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than tamsulosin obtained from the sample solution is not larger than 1/2 times the peak area of tamsulosin from the standard solution.

Operating conditions—

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

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m 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 for liquid chromatography.

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

Time span of measurement: Until tamsulosin is eluted, beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 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 $10 \,\mu$ L of this solution is equivalent to 1.4 to 2.6% of that from $10 \,\mu$ L of the standard solution.

System performance: Dissolve 5 mg of Tamsulosin Hydrochloride and 10 mg of propyl parahydroxybenzoate 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 $10 \,\mu$ L of this solution under the above operating conditions, tamsulosin and propyl parahydroxybenzoate are eluted in this order with the resoluton between these peaks being not less than 12.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ 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%.

(ii) Perform the test with 10 μ L each of the sample solution and standard solution which are obtained in above (i) as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than tamsulosin obtained from the sample solution is not larger than 1/2 times the peak area of tamsulosin from the standard solution.

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Purity (2) (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 for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of tamsulosin is about 2.5 minutes.

Time span of measurement: About 5 times as long as the retention time of tamsulosin, beginning after the peak of tamsulosin.

System suitability-

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase used in the Purity (2) (i) to make exactly 50 mL. Confirm that the peak area of tamsulosin obtained from $10 \,\mu$ L of this solution is equivalent to 1.4 to 2.6% of that from $10 \,\mu$ L of the standard solution.

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System performance: Proceed as directed in the system suitability in the Purity (2) (i).

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ 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%.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 immediately titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

> Each mL of 0.1 mol/L perchloric acid VS = $44.50 \text{ mg of } C_{20}H_{28}N_2O_5S.HCl$

Containers and storage Containers-Well-closed containers.

Tamsulosin Hydrochloride Extended-release Tablets

タムスロシン塩酸塩徐放錠

Tamsulosin Hydrochloride Extended-release Tablets contain not less than 94.0% and not more than 106.0% of the labeled amount of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S$.HCl: 444.97).

Method of preparation Prepare as directed under Tablets, with Tamsulosin Hydrochloride.

Identification To an amount of powdered Tamsulosin Hydrochloride Extended-release Tablets, equivalent to 1 mg of Tamsulosin Hydrochloride according to the labeled amount, add about 5 g of porcelain balls with about 5 mm in diameter, add 20 mL of 0.2 mol/L sodium hydroxide TS, warm at 50°C for 10 minutes, and shake vigorously for 15 minutes. Then, add 7 mL of acetonitrile, shake slightly, and centrifuge. Take the supernatant liquid, add 2.5 g of sodium chloride and 5 mL of ethyl acetate, shake vigorously for 5 minutes, and centrifuge. Take the supernatant liquid, evaporate to dryness at 50°C in a water bath, dissolve the residue with 20 mL of water, and filter through a membrane filter with a pore size not exceeding $0.45 \,\mu\text{m}$. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 222 nm and 226 nm, and between 278 nm and 282 nm.

Uniformity of dosage units $\langle 6.02 \rangle$ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Tamsulosin Hydrochloride Extended-release Tablets add about 5 g of porcelain balls with about 5 mm in diameter and 5 mL of water, and shake to disintegrate the tablet. Add 20 mL of a solution of sodium hydroxide (1 in 500), warm at 50° C for 10 minutes, shake vigorously for 30 minutes, and add 10 mL of acetonitrile and 5 mL of 0.2 mol/L hydrochloric acid TS. To this solution add exactly 5 mL of the internal standard solution for every 0.1 mg of tamsulosin hydrochloride, add the mobile phase to make 50 mL, shake slightly, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μ m. To V mL of the filtrate add the mobile phase to make V' mL so that each mL contains about 2 μ g of tamsulosin hydrochloride (C₂₀H₂₈N₂O₅S.HCl), and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S.HCl$) = $M_S \times Q_T/Q_S \times V'/V \times 1/100$

 $M_{\rm S}$: Amount (mg) of tamsulosin hydrochloride for assay

Internal standard solution—A solution of methyl parahydroxybenzoate in the mobile phase (1 in 25,000).

Dissolution Being specified separately.

Assay Weigh accurately the mass of not less than 20 Tamsulosin Hydrochloride Extended-release Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 mg of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S$. HCl), add about 5 g of porcelain balls with about 5 mm in diameter and 5 mL of water, shake, then add 20 mL of a solution of sodium hydroxide (1 in 500), warm at 50°C for 10 minutes, and shake vigorously for 30 minutes. To this solution add 10 mL of acetonitrile, 5 mL of 0.2 mol/L hydrochloric acid TS and exactly 5 mL of the internal standard solution, then add 5 mL of the mobile phase, shake slightly, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μ m, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of tamsulosin hydrochloride for assay, previously dried at 105°C for 2 hours, and dissolve in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of tamsulosin to that of the internal standard.

> Amount (mg) of tamsulosin hydrochloride ($C_{20}H_{28}N_2O_5S.HCl$) = $M_S \times Q_T/Q_S \times 1/100$

 $M_{\rm S}$: Amount (mg) of tamsulosin hydrochloride for assay

Internal standard solution—A solution of methyl parahydroxybenzoate in the mobile phase (1 in 25,000). *Operating conditions*—

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

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m 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 to pH 2.0 with sodium hydroxide TS, and add water to make 1000

mL. To 700 mL of this solution add 300 mL of acetonitrile for liquid chromatography.

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

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the internal standard and tamsulosin 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 $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of tamsulosin to that of the internal standard is not more than 1.0%.

Containers and storage Containers-Well-closed containers.

Tannic Acid

タンニン酸

Tannic Acid is the tannin usually obtained from nutgalls or rhusgalls.

Description Tannic Acid occurs as a yellowish white to light brown, amorphous powder, glistening leaflets, or spongy masses. It is odorless or has a faint, characteristic odor, and has a strongly astringent taste.

It is very soluble in water and in ethanol (95), and practically insoluble in diethyl 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 develops. 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 filter the solution. To 5 mL of the filtrate add 5 mL of ethanol (95): no turbidity is produced. Add further 3 mL of diethyl 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.

Loss on drying $\langle 2.41 \rangle$ Not more than 12.0% (1 g, 105°C, 2 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 1.0% (0.5 g).

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Tartaric Acid

酒石酸



C₄H₆O₆: 150.09 (2*R*,3*R*)-2,3-Dihydroxybutanedioic acid [87-69-4]

Tartaric Acid, when dried, contains not less than 99.7% of $C_4H_6O_6$.

Description Tartaric Acid occurs as colorless crystals or a white, crystalline powder. It is odorless, and has a strong acid taste.

It is very soluble in water, freely soluble in ethanol (95), and slightly soluble in diethyl ether.

A solution of Tartaric Acid (1 in 10) is dextrorotatory.

Identification (1) Ignite Tartaric Acid gradually: it decomposes and an odor of burning sugar is perceptible.

(2) A solution of Tartaric Acid (1 in 10) changes blue litmus paper to red, and responds to the Qualitative Tests $\langle 1.09 \rangle$ for tartrate.

Purity (1) Sulfate $\langle 1.14 \rangle$ —Perform the test with 0.5 g of Tartaric Acid. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(2) Oxalate—Dissolve 1.0 g of Tartaric Acid in 10 mL of water, and add 2 mL of calcium chloride TS: no turbidity is produced.

(3) Heavy metals <1.07>—Proceed with 2.0 g of Tartaric Acid 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).

(4) Calcium—Neutralize a solution of 1.0 g of Tartaric Acid in 10 mL of water with ammonia TS, and add 1 mL of ammonium oxalate TS: no turbidity is produced.

(5) Arsenic $\langle 1.11 \rangle$ —Prepare the test solution with 2.0 g of Tartaric Acid according to Method 1, and perform the test (not more than 1 ppm).

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (3 g, silica gel, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.05% (1 g).

Assay Weigh accurately about 1.5 g of Tartaric Acid, previously dried, dissolve in 40 mL of water, and titrate $\langle 2.50 \rangle$ with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

> Each mL of 1 mol/L sodium hydroxide VS = $75.05 \text{ mg of } C_4H_6O_6$

Containers and storage Containers-Well-closed containers.

Taurine

タウリン

H₂N∽

C₂H₇NO₃S: 125.15 2-Aminoethanesulfonic acid [*107-35-7*]

Taurine, when dried, contains not less than 99.0% and not more than 101.0% of $C_2H_7NO_3S$.

SO₃H

Description Taurine occurs as colorless or white crystals, or a white crystalline powder.

It is soluble in water, and practically insoluble in ethanol (99.5).

The pH of a solution prepared by dissolving 1.0 g of Taurine in 20 mL of freshly boiled and cooled water is between 4.1 and 5.6.

Identification Determine the infrared absorption spectrum of Taurine as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Clarity and color of solution—A solution obtained by dissolving 1.0 g of Taurine in 20 mL of water is clear and colorless.

(2) Chloride $\langle 1.03 \rangle$ —Perform the test with 1.0 g of Taurine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(3) Sulfate $\langle 1.14 \rangle$ —Perform the test with 2.0 g of Taurine. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010%).

(4) Ammonium $\langle 1.02 \rangle$ —Perform the test with 0.25 g of Taurine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <1.07>—Proceed with 2.0 g of Taurine 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 $\langle 1.10 \rangle$ —Prepare the test solution with 2.0 g of Taurine 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 10 ppm).

(7) Related substances—Dissolve 1.0 g of Taurine in 50 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 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 $\langle 2.03 \rangle$. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of water, ethanol (99.5), 1-butanol and acetic acid (100) (150:150:100:1) to a distance of about 10 cm, and airdry the plate. Spray evenly ninhydrin-butanol TS on the plate, and heat at 105°C for 5 minutes: the spot other than the principle spot with the sample solution is not more than one spot, and it is not more intense than the spot with the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.20% (1 g, 105°C, 2 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Taurine, previously dried, dissolve in 50 mL of water, add 5 mL of formaldehyde solution, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

> Each mL of 0.1 mol/L sodium hydroxide VS = $12.52 \text{ mg of } C_2H_7NO_3S$

Containers and storage Containers—Well-closed containers.

Tazobactam

タゾバクタム



 $C_{10}H_{12}N_4O_5S:$ 300.29 (2*S*,3*S*,5*R*)-3-Methyl-7-oxo-3-(1*H*-1,2,3-triazol-1-ylmethyl)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide [*89786-04-9*]

Tazobactam contains not less than 980 μ g and not more than 1020 μ g (potency) per 1 mg, calculated on the anhydrous basis. The potency of Tazobactam is expressed as mass (potency) of C₁₀H₁₂N₄O₅S.

Description Tazobactam occurs as a white to pale yellowish white, crystalline powder.

It is freely soluble in dimethylsulfoxide and in N,Ndimethylformamide, and slightly soluble in water, in methanol and in ethanol (99.5).

It dissolves in a solution of sodium hydrogen carbonate (3 in 100).

Identification (1) Determine the infrared absorption spectrum of Tazobactam as directed in the potassium bromide disk method under the Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Tazobactam RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Determine the ¹H spectrum of a solution of Tazobactam in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 35) as directed under the Nuclear Magnetic Resonance Spectroscopy $\langle 2.21 \rangle$, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around δ 1.3 ppm, and double signals, B and C, at around δ 7.8 ppm and at around δ 8.1 ppm. The ratio of the integrated intensity of each signal, A:B:C, is about 3:1:1.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_D^{20}$: +162 - +167° (1 g calculated on the anhydrous basis, *N*,*N*-dimethylformamide, 100 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Tazobactam in 10 mL of sodium hydrogen carbonate (3 in 100): the solution is clear. Perform the test with the solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: the absorbance at 420 nm is not more than 0.14.

(2) Heavy metals $\langle 1.07 \rangle$ —Proceed with 1.0 g of Tazobactam 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-This operation must be performed quickly. Dissolve 50 mg of Tazobactam in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 1 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 exactly 50 μ L each of the sample solution, the standard solutions (1) and (2) as directed under the Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the area of the peak, having the relative retention time of about 0.17 with respect to tazobactam, obtained from the sample solution is not larger than 4/5 times the peak area of tazobactam from the standard solution (1), the area of the peak other than the peak of tazobactam and the peak having the relative retention time of about 0.17 with respect to tazobactam from the sample solution is not larger than the peak area of tazobactam from the standard solution (2), and the total area of the peaks other than the peak of tazobactam and the peak having the relative retention time of about 0.17 with respect of tazobactam from the sample solution is not larger than 2 times the peak area of tazobactam from the standard solution (2).

Operating conditions—

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

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

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution (1), and add the mobile phase to make exactly 20 mL. Confirm that the peak area of tazobactam obtained from 50 μ L of this solution is equivalent to 3 to 7% of that of tazobactam from 50 μ L of the standard (1).

System performance: When the procedure is run with 50 μ L of the standard solution (1) under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of tazobactam are not less than 2000 and 0.8 – 1.2, respectively.

System repeatability: When the test is repeated 6 times with $50 \,\mu\text{L}$ of the standard solution (1) under the above operating conditions, the relative standard deviations of the peak area of tazobactam is not more than 1.0%.

(4) Residual solvent—Being specified separately.

Water $\langle 2.48 \rangle$ Not more than 0.5% (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 $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Bacterial endotoxins <4.01> Less than 0.04 EU/mg (potency).

Assay Weigh accurately an amount of Tazobactam and Tazobactam RS, equivalent to about 50 mg (potency), dissolve each in exactly 10 mL of the internal standard solution, add water to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μ L each of the sample solution and standard solution as directed under the Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak areas of tazobactam to that of the internal standard.

Amount [μ g (potency)] of tazobactam (C₁₀H₁₂N₄O₅S) = $M_{\rm S} \times (Q_{\rm T}/Q_{\rm S}) \times 1000$

 $M_{\rm S}$: Amount [mg (potency)] of Tazobactam RS

Internal standard solution—A solution of phenylalanine (1 in 400).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 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 (10 μ m in particle diameter).

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

Mobile phase: Dissolve 1.32 g of diammonium hydrogen phosphate in 750 mL of water, adjust the pH to 2.5 with phosphoric acid, add water to make 1000 mL, and add 25 mL of acetonitrile.

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

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the internal standard and tazobactam 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 $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of tazobactam to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Expiration date 24 months after preparation.

Teceleukin (Genetical Recombination)

テセロイキン(遺伝子組換え)

MAPTSSSTKK TQLQLEHLLL DLQMILNGIN NYKNPKLTRM LTFKFYMPKK ATELKHLQCL EEELKPLEEV LNLAQSKNFH LRPRDLISNI NVIVLELKGS

C₆₉₈H₁₁₂₇N₁₇₉O₂₀₄S₈: 15547.01 [*136279-32-8*]

The desired product of Teceleukin (Genetical Recombination) is a protein consisting of 134 amino acid residues manufactured by *E. coli* through expression of human interleukin-2 cDNA.

It is a solution and possesses a T-lymphocyte activating effect.

It contains potency between 7.7×10^6 and 1.54×10^7 units per mL, and not less than 7.7×10^6 units per mg of protein.

Description Teceleukin (Genetical Recombination) occurs as a clear and colorless liquid.

Identification (1) Measure accurately an appropriate amount of Teceleukin (Genetical Recombination), add accurately to a concentration of 200 units per mL of culture medium for assay of teceleukin, and use this solution as the sample stock solution. Dilute reference anti-interleukin-2 antibody for teceleukin with culture medium for assay of teceleukin to a concentration of approximately 200 neutral units per mL and use this solution as the interleukin-2 neutral antibody solution. Accurately add an equivalent volume of the interleukin-2 neutral antibody solution to the sample stock solution, shake, and then leave for 1 hour in a 37°C incubator in air containing 5% carbon dioxide. This solution is the sample solution. Prepare a standard solution by accurately adding an equivalent volume of culture medium for assay of teceleukin to the sample stock solution, mixing, and then processing in the same way. Process the sample and standard solutions according to the assay method, determine their respective dilution coefficients, $D_{\rm N}$ and $D_{\rm T}$, and then calculate the neutralization rate, which should be at least 90%, using the following formula.

Neutralization rate (%) = $(D_{\rm T} - D_{\rm N})/D_{\rm T} \times 100$

However, please note if the mean values of the absorbance of the maximum uptake control solution and absorbance of the minimum uptake control solution do not fit the standard curve, the neutralization coefficient is to be determined within the following range.

Neutralization coefficient (%) > $(D_T - 2)/D_T \times 100$

(2) Place a volume of Teceleukin (Genetical Recombination) corresponding to approximately $50 \mu g$ of protein into 2 test tubes for hydrolysis, evaporate to dryness under vacuum, and use one as the sample (1). To the other, add $50 \mu L$ of a mixture of formic acid and hydrogen peroxide (30) (9:1) that has been left at room temperature for one hour, cool for 4 hours in ice, add 0.5 mL of water, and then evaporate to dryness under vacuum to give the sample (2). To 1.3 mL of methanesulfonic acid add 3.7 mL of water, mix well, add and dissolve 10 mg of 3-(2-aminoethyl)indole, to make a 4 mol/L methanesulfonic acid solution. Dissolve 39.2 g of trisodium citrate dihydrate, 33 mL of hydrochloric acid, 40 mL of thiodiglycol, and 4 mL of lauromacrogol solution (1 in 4) in 700 mL of water, adjust the pH to 2.2, add water to 1000 mL, add 100 μ L of capric acid, and mix to make a sodium citrate solution for dilution. Add 50 μ L of freshly prepared 4 mol/L methanesulfonic acid to the sample (1) and sample (2), cool to -70° C, and then deaerate under vacuum. Heat to $115^{\circ}C \pm 2^{\circ}C$ for 24 hours after sealing these test tubes under reduced pressure. After cooling, unseal, add $50 \,\mu\text{L}$ of 4 mol/L sodium hydroxide TS followed by 0.4 mL of sodium citrate solution for dilution to make the sample solution (1) and sample solution (2). Separately, accurately measure 0.25 mmol amounts of L-aspartic acid, L-threonine, L-serine, L-glutamic acid, L-proline, glycine, L-alanine, Lvaline, L-methionine, L-isoleucine, L-leucine, L-tyrosine, Lphenylalanine, L-lysine hydrochloride, ammonium chloride, L-histidine hydrochloride monohydrate, and L-arginine hydrochloride as well as 0.125 mmol of L-cysteine and then dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. This is the amino acid standard stock solution. Accurately measure 1 mL of this solution, and add sodium citrate solution for dilution to make exactly 25 mL. This is solution A. Accurately weigh approximately 20 mg of Ltryptophan and dissolve in water to make exactly 1000 mL. This is solution B. Accurately measure 10 mL of both solution A and solution B, combine together, and add sodium citrate solution for dilution to make exactly 50 mL. This is the amino acid standard solution. Separately, accurately weigh approximately 17 mg of L-cysteic acid and dissolve in sodium citrate solution for dilution to make exactly 50 mL. Accurately measure 1 mL of this solution and add sodium citrate solution for dilution to make exactly 100 mL. This is the cysteic acid standard solution. Accurately measure 0.25 mL of the sample solution (1), the sample solution (2), amino acid standard solution, and the cysteic acid standard solution. When the test is conducted by Liquid Chromatography $\langle 2.01 \rangle$ under the following conditions, peaks for the 18 amino acids are observed in the chromatogram obtained from the sample solution (1). Also, measure the peak area of each amino acid in the sample solution (1) and the amino acid standard solution, and taking the molar number of alanine in the sample solution (1) as 5.0, determine the concentrations of aspartic acid, glutamic acid, proline, glycine, methionine, leucine, tyrosine, phenylalanine, lysine, histidine, tryptophan, and arginine and then calculate the molar ratio for each amino acid. Also, measure the cysteic acid peak areas of the sample solution (2) and the cysteic acid standard solution, determine the concentration of cysteine, and, taking the molar number of alanine in the sample solution (2) as 5.0, calculate the molar ratio of cysteine. When determining the molar ratios of the respective amino acids, aspartic acid is 11.4 to 12.6, glutamic acid 17.1 to 18.9, proline 4.5 to 5.5, glycine 1.8 to 2.2, cysteine 2.7 to 3.3, methionine 4.5 to 5.5, leucine 20.9 to 23.1, tyrosine 2.7 to 3.3, phenylalanine 5.4 to 6.6, lysine 10.5 to 11.6, histidine 2.7 to 3.3, tryptophan 0.7 to 1.2, and arginine 3.6 to 4.4. Operating conditions—

Detector: Visible absorption photometer [wavelengths: 440 nm (proline) and 570 nm (amino acids other than proline)] Column: A stainless steel column with an inside diameter of 4 mm and length of 25 cm packed with a strongly acidic ion exchange resin for liquid chromatography consisting of polystyrene to which sulphonate group binds.

Column temperature: A constant temperature of about 50° C when the sample is injected. After a certain time, increase the temperature to a constant temperature of about 62° C.

Reaction temperature: A constant temperature of about $98\,^\circ\text{C}$.

Time for color formation: Approximately 2 minutes.

Mobile phase: After preparing mobile phases A, B, and C according to the following table, add 0.1 mL of capric acid to each.

	Mobile phase A	Mobile phase B	Mobile phase C
Citric acid monohydrate	18.70 g	10.50 g	7.10 g
Trisodium citrate dihydrate	7.74 g	14.71 g	26.67 g
Sodium chloride	7.07 g	2.92 g	54.35 g
Ethanol (99.5)	60 mL	_	_
Benzyl alcohol	_	_	10 mL
Thiodiglycol	5 mL	5 mL	_
Lauromacrogol solution (1 in 4)	4 mL	4 mL	4 mL
Water	Appropriate amount	Appropriate amount	Appropriate amount
pH	3.2	4.3	4.7
Total volume	1000 mL	1000 mL	1000 mL

Changing mobile phases and column temperature: When operating under the above conditions using 0.25 mL of amino acid standard solution, the amino acids will elute in the following order; aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, ammonia, histidine, tryptophan, and arginine. Switchover to mobile phase A, mobile phase B, and mobile phase C, in sequence so that the resolution between the peaks of cystine and valine is 2.0 or more and that between ammonia and histidine is 1.5 or more. Also, increase the temperature after a constant length of time so that the resolution between the peaks of glutamic acid and proline is at least 2.0.

Reaction reagents: Dissolve 408 g of lithium acetate dihydrate in water, and add 100 mL of acetic acid (100) and water to make 1000 mL. To this solution add 1200 mL of dimethylsulfoxide and 800 mL of 2-methoxyethanol. This is solution (I). Separately, mix together 600 mL of dimethylsulfoxide and 400 mL of 2-methoxyethanol and then add 80 g of ninhydrin and 0.15 g of sodium borohydride. This is solution (II). After gassing 3000 mL of the solution (I) for 20 minutes with nitrogen, rapidly add 1000 mL of the solution (II) and then mix by gassing for 10 minutes with nitrogen.

Mobile phase flow rate: About 0.275 mL every minute.

Reaction reagent flow rate: About 0.3 mL every minute. *System suitability—*

System performance: When 0.25 mL of the amino acid standard solution is run under the above conditions, the resolution between the peaks of threonine and serine is at least 1.5.

(3) Dissolve 0.242 g of 2-amino-2-hydroxymethyl-1,3propanediol, 5.0 g of sodium lauryl sulfate, and 74 mg of disodium dihydrogen ethylenediamine tetraacetate dihydrate

in 60 mL of water. After adjusting the pH to 8.0 using 1 mol/L hydrochloric acid TS, add water to make 100 mL. This is the molecular weight determination buffer solution. Accurately measure 20 μ L of Teceleukin (Genetical Recombination), add exactly 20 µL of the molecular weight determination buffer solution and $2 \mu L$ of 2-mercaptoethanol, and then heat for 5 minutes on a 90 to 100°C water bath without allowing any water evaporation from the mixture. After cooling, add exactly $1 \mu L$ of bromophenol blue solution (1 in 2000) and then shake. This is the sample solution. Separately, accurately measure $5 \mu L$ of molecular weight marker for teceleukin, and add exactly 50 μ L of water, 55 μ L of the molecular weight determination buffer solution, and 5 μ L of 2-mercaptoethanol, and then heat for 5 minutes on a 90 to 100°C water bath without allowing any water evaporation from the mixture. After cooling, add exactly $1 \,\mu L$ of bromophenol blue solution (1 in 2000), and shake well. This is the molecular weight standard solution. When conducting a test using SDS-polyacrylamide gel electrophoresis with 1 μ L each of the sample solution and the molecular weight standard solution, the molecular weight of the main band is between the range of 14,000 and 16,000.

Operating conditions-

Equipment: Horizontal electrophoresis vessel equipped with a cooling unit, a device that accumulates load voltage over time, and a direct current power source device that controls the amperage, voltage, wattage.

Spotting of solutions: Solutions are spotted on concentrating gel of polyacrylamide gel sheets.

Electrophoresis conditions

Polyacrylamide gel sheet: Polyester sheet to which a polyacrylamide gel (width, about 43 mm, length, about 50 mm, and thickness, about 0.5 mm) is closely adhered. The polyacrylamide gel consists of a concentrating gel with a gel support concentration of 7.5% and a 3% degree of crosslinking and a separating gel with corresponding values of 20% and 2%. The gel contains pH 6.5 Trisacetate buffer.

Buffer solution for electrode: Prepared by dissolving 35.83 g of tricine, 24.23 g of 2-amino-2-hydroxymethyl-1,3-propanediol, and 5.5 g of sodium lauryl sulfate in water to make 1000 mL.

Cooling temperature of gel support plate: 15°C. Running conditions

Pre-electrophoresis and electrophoresis: The voltage, amperage, and wattage should not exceed 250 V, 10 mA, and 3 W, respectively. The amperage and wattage should be proportional to the number of polyacrylamide sheets.

Immediately after adding sample: The voltage, amperage, and wattage should not exceed 250 V, 1 mA, and 3 W, respectively. The amperage and wattage should be proportional to the number of polyacrylamide sheets. Electrophoresis time

Before adding sample: Until value of load voltage integrated with respect to time reaches 60 V \cdot h.

Immediately after adding sample: Until value of load voltage integrated with respect to time reaches $1 \text{ V} \cdot \text{h}$.

Main electrophoresis: Until value of load voltage integrated with respect to time reaches 140 V \cdot h. Fixation and staining

Dissolve 25 g of anhydrous sodium carbonate anhydride and 0.8 mL of formaldehyde solution in water to make 1000 mL. This is the developing solution. After immersing the polyacrylamide gel sheet in a mixture of ethanol (99.5), water and acetic acid (100) (5:4:1) for 2 minutes, immerse for 2 minutes in a mixture of water, ethanol (99.5) and acetic acid (100) (17:2:1). Change the mixture, immerse for another 4 minutes, immerse in water for 2 minutes to rinse the polyacrylamide gel sheet, and change the water to immerse for 2 minutes. This procedure is carried out with heating to 50°C. Next, while heating at 40°C, immerse for 10 to 15 minutes in diluted silver nitrate TS (1 in 7), warm to 30°C, and gently rinse the polyacrylamide gel sheet with water. While warming at 30°C, immerse the polyacrylamide gel sheet in freshly prepared developing solution. After obtaining adequate color formation, immerse the polyacrylamide gel sheet in diluted acetic acid (100) (1 in 20) to terminate the color formation. Estimation of molecular weight

Plot graphs for each band obtained from the molecular weight standard solution, distance from the border of the concentrating gel and separating gel, and the logarithm of the molecular weight of proteins in each band. Calculate the molecular mass by reading the corresponding position of the major band obtained from the sample solution on the graph.

(4) The isoelectric point determined from the electrophoresis position is 7.4 to 7.9 when $3 \mu L$ of Teceleukin (Genetical Recombination) and $8 \mu L$ of isoelectric marker for teceleukin are tested by the polyacrylamide gel isoelectric method.

Operating conditions—

Equipment: Horizontal electrophoretic vessel with cooling unit and direct current power source that can perform constant wattage control.

Preparation of polyacrylamide gel: Dissolve 1.62 g of polyacrylamide and 50 mg of N, N'-methylenebisacrylamide in water to make 25 mL. Accurately measure 7.5 mL of this solution, 2 mL of a 10 mL solution prepared by adding water to 5 g of glycerin, and 0.64 mL of a pH 3 to pH 10 amphoteric electrolyte solution, and degas under reduced pressure while stirring thoroughly. Next, accurately measure 74 μ L of freshly prepared ammonium peroxodisulfate solution (1 in 50), 3μ L of N, N, N', N'-tetramethylethylenediamine, and 50 μ L of freshly prepared riboflavin sodium phosphate solution (1 in 1000), stir well, immediately pour on a gel preparation plate (10 cm wide, 11 cm long, and 0.8 mm thick), and then expose to a fluorescent light source for 60 minutes to gelate.

Spotting

Add Teceleukin (Genetical Recombination) or isoelectric marker for teceleukin 30 minutes after starting electrophoresis to wells in gel plates to which plastic tape (3.5 mm wide, 3.5 mm long, 0.4 mm thick) has been applied in advance and that have undergone gelation.

Electrophoresis conditions

Cathode solution: Sodium hydroxide TS.

Anode solution: DL-aspartic acid solution (133 in 25,000).

Cooling temperature of gel support plate: $2 \pm 1^{\circ}$ C.

Running conditions: After starting the electrophoresis, a constant wattage of 10 W for 20 minutes and 20 W thereafter. However, the voltage should be 3000 V or less.

Running time: 120 to 140 minutes while blowing Nitrogen into the electrophoresis vessel.

Fixation and washing

Dissolve 28.75 g of trichloroacetic acid and 8.65 g of 5sulfosalicylic acid dihydrate in 75 mL of methanol and 175 mL of water. Immerse the gel in this solution for 60 minutes to fix the protein to the gel. After fixation, immerse for 10 minutes in a mixture of water, ethanol (99.5) and acetic acid (100) (67:25:8).

Staining and decolorization

Dissolve 0.11 g of Coomassie brilliant blue G-250 in 25 mL of ethanol (99.5), and add 8 mL of acetic acid (100) and water to make 100 mL. This is the staining solution. Immerse the gel for 10 minutes while heating at 60° C in freshly filtered staining solution. After staining, decolorize by immersing in a mixture of water, ethanol (99.5) and acetic acid (100) (67:25:8).

Determination of isoelectric point

Plot the protein isoelectric points and the distance from the cathode of each band obtained from the isoelectric markers for teceleukin. Calculate the isoelectric point from the corresponding position of the major bands obtained from the sample solution.

pH <2.54> 2.7 - 3.5

Purity (1) Host cell-derived protein-Take an appropriate amount of Teceleukin (Genetical Recombination) and add an exact amount of diluted acetic acid (100) (1 in 350) to make a solution containing between 0.68 and 0.72 mg of protein in one mL. This is the sample stock solution. Dissolve 1.52 g of 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride and 10.94 g of 2-amino-2-hydroxymethyl-1,3propanediol in water to make 200 mL. Dissolve 0.5 g of bovine serum albumin in 25 mL of this solution. This is 2 w/v% bovine serum albumin-Tris-hydrochloride buffer solution. Accurately measure 0.5 mL of the sample stock solution, add exactly $30 \,\mu L$ of sodium carbonate TS, stir, and immediately add exactly 0.47 mL of the 2 w/v% bovine serum albumin-Tris-hydrochloride buffer solution to make the sample solution. Accurately measure 10 mL of dilute acetic acid (100) (1 in 350), add 0.6 mL of sodium carbonate TS, and then add 2 w/v% bovine serum albumin-Trishydrochloride buffer solution to make exactly 20 mL. This is the dilution solution. Add the E. coli protein stock solution to this dilution solution to make a solution containing 0.015 μ g of E. coli protein in one mL. This is standard solution (1). Accurately dilute this solution serially two-fold with the dilution solution to make standard solutions (2) to (8) having different concentrations of E. coli protein. Dissolve 0.5 g of bovine serum albumin in 100 mL of 0.01 mol/L phosphate buffer-sodium chloride TS, pH 7.4. This is the wash solution. Accurately measure 0.1 mL of the sample solution, standard solutions (1) to (8), and dilution solution as a blank standard solution and place each in 3 wells in solid phase plates (place dilution solution in 6 wells), cover with plastic wrap, mix by shaking in a horizontal direction, and then leave standing at a constant temperature of about 25°C for 5 to 16 hours. Next, remove the solution from each well by aspiration, add 0.25 mL of the the wash solution, mix again by shaking in a horizontal direction, and then remove by aspiration. Repeat this procedure 2 more times by adding 0.25 mL of the wash solution to each well. Freshly dilute peroxidase marker antibody stock solution with 1 w/v% bovine serum albumin-phosphate buffer-sodium chloride TS, add exactly 0.1 mL to each well, cover with plastic wrap, mix by shaking in a horizontal direction, and then leave standing at a constant temperature of about 25°C for 16 to 24 hours. Next, remove the solution in the wells by aspiration, add 0.25 mL of the wash solution, mix by shaking in a horizontal direction, and then remove the solution by aspiration. Using 0.25 mL of the wash solution, repeat this procedure 2 more times for each well. To each well, accurately add 0.1 mL of teceleukin chromophore solution, stir gently, and then shield from light and leave standing for 30 minutes at a constant temperature of about 25°C. Add exactly 0.1 mL of diluted sulfuric acid (3 in 50) to each well and then mix by gently shaking horizontally. Measure the absorbances of these solutions A_{T2} and A_{S2} at 450 nm and A_{T1} and A_{S1} at 510 nm. Prepare a standard curve by plotting the values obtained from each standard solution $(A_{s2} - A_{s1})$ on a graph having the concentration of E. coli protein (ng/mL) in logarithmic scale on the horizontal axis and the absorbance values on the vertical axis. Match the values obtained from the sample solution $(A_{T2} - A_{T1})$ to the standard curve, determine the concentration A of E. coli protein in the sample solution, and take the mean. The amount of E. coli protein is not more than 5 ng when the amount of E. coli protein per mg of protein is determined using the following formula.

Amount (ng) of E. coli-derived protein per mg protein = A/C

C: Protein concentration (mg/mL) in sample solution

The test is valid if the E. coli protein concentration is 0.3 ng/mL or less when the concentration is obtained by fitting the absorbance value at detection limit, calculated from the following formula using absorvance value of the dilution solution, to the standard curve.

Absorbance at detection limit

$$= \bar{X} + 3.3 \times \sqrt{\left\{\sum_{i=1}^{6} (X_i - \bar{X})^2\right\}/(6-1)}$$

- X_i : Individual absorbance values obtained from the dilution solution
- \bar{X} : The mean of absorbance values obtained from the dilution solution
- 6: The number of wells in the microplate containing dilution solution

(2) Tetracycline hydrochloride-Serially subculture through 2 passages at 35 to 37°C the test bacteria Micrococcus luteus ATCC9341 in a slant culture of test bacteria inoculation media for teceleukin and then dilute this 100-fold by adding sterilized purified water. This is the test bacteria solution. Store the test bacteria solution at 5°C or less and use the solution within 5 days. Dilute the test bacteria solution serially by adding sterilized purified water, add an appropriate amount to 100 mL of normal agar medium for teceleukin, conduct a preliminary test, and determine the amount of tetracycline hydrochloride that shows an inhibition zone corresponding to standard solution containing 0.5 μ g (potency) in 1 mL. Add this amount to 100 mL of normal agar medium for teceleukin dissolved and then cooled to 45 to 50°C and mix. Pipet 25 mL of this solution into square Petri dishes $(135 \times 95 \text{ mm})$ and spread horizontally to solidify. Prepare plates for testing by making an appropriate number of wells in this agar medium. The volume of the test bacteria solution to which 100 mL of normal agar medium for teceleukin has been added is 0.25 to 1.0 mL. Accurately

measure an appropriate amount of Tetracycline Hydrochloride RS and dilute accurately with water to make a clear solution with a concentration of 1 mg (potency)/mL. Accurately measure an appropriate amount of this solution and dilute precisely with water to make standard solutions with concentrations of 4, 2, 1 and $0.5 \,\mu g$ (potency)/mL. Separately, dilute Teceleukin (Genetical Recombination) with diluted acetic acid (100) (3 in 1000) if needed, or alternatively concentrate under reduced pressure, to make a sample solution with a protein concentration of 0.8 to 1.2 mg/mL. Accurately measure $25 \,\mu\text{L}$ of the sample solution and each standard solution, and add each to the wells in the same test plate. Repeat the same procedure for at least 3 more test plates. Leave the test plates at room temperature for 30 to 60 minutes and then incubate for 16 to 18 hours at 35 to 37°C. Measure the inhibitions zones to a diameter of 0.25 mm. Determine the mean among the test plates for each of the solutions.

Prepare a standard curve by plotting a graph with the concentration of each standard solution in logarithmic scale on the horizontal axis and the diameter of the inhibition zone on the vertical axis. Match the diameter of the inhibition zone of teceleukin from the standard curve and determine A, the concentration of tetracycline hydrochloride. When the amount of tetracycline hydrochloride per mg of protein is determined by the following formula, the amount is not more than 0.7 μ g. However, if an inhibition zone is not seen, or is seen but the diameter is smaller than 0.5 μ g/mL on the standard curve, A is taken as being 0.5 μ g/mL or less.

Amount [μ g (potency)] of tetracycline hydrochloride (C₂₂H₂₄N₂O₈.HCl) per mg of protein = A/P

P: The protein concentration (mg/mL) of the sample solution.

(3) Desmethionyl form—Add water to an appropriate amount of teceleukin to make a sample solution with a protein concentration of about 0.17 mg/mL. Perform the test with 1.2 mL of this solution as directd under Liquid Chromatography $\langle 2.01 \rangle$ under the following conditions. Determine using automatic integration the peak area, A_2 , of teceleukin and the peak area of the desmethionyl form with a relative retention time of about 0.8 relative to teceleukin, A_1 . The content of the desmethionyl form is not more than 1.0% when determined using the following formula.

Amount (%) of desmethionyl form = $A_1/(A_1 + A_2) \times 100$

Operating conditions-

Detector: Ultravioletabsorption photometer (wavelength: 280 nm)

Columns: Two stainless steel columns with inside diameters of 7.5 mm and lengths of 7.5 cm connected in sequence and packed with $10 \,\mu$ m synthetic polymer bound to diethylaminoethyl base for liquid chromatography.

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

Mobile phase A: Mix 0.658 g of diethanolamine in 400 mL of water, adjust the pH to 9.0 by adding 1 mol/L hydrochloric acid TS, and then add water to make 500 mL.

Mobile phase B: Add 300 mL of water to 2.6 mL of a pH 6 to 9 amphoteric electrolyte solution and 0.5 mL of a pH 8 to 10.5 amphoteric electrolyte solution, adjust to pH 7 with diluted hydrochloric acid (9 in 100), and then add water to

make 400 mL.

Switching mobile phases and sample injection: Inject the sample solution while running the mobile phase A. Repeatedly inject 10 times a sample solution volume of 0.11 mL followed by a single injection of $100 \,\mu$ L. After injecting the entire volume and running mobile phase A for 60 minutes, switch to mobile phase B. After measuring the sample solution and after running 1 mol/L sodium chloride TS for 10 minutes for postreatment and cleaning of the columns, inject $100 \,\mu$ L of sodium hydroxide TS while running the mobile phase A and then 55 minutes later start injection of the next sample solution.

Flow: Adjust the flow of the mobile phase B so that the retention time for teceleukin is 45 to 65 minutes. Measure the retention time from the point at which the mobile phase is switched to the mobile phase B.

System suitability-

System performance: Dissolve in water a mixture of two kinds of equine heart-derived myoglobin whose isoelectric points are 6.76 and 7.16 to make a concentration of approximately 0.5 mg/mL. Mix together 50 μ L of this solution, 50 μ L of Teceleukin (Genetical Recombination), and 1.47 mL of water. When 1.2 mL of this solution is run under the above conditions, myoglobin and teceleukin are eluted in this order, and their respective peaks are completely separated.

(4) Dimer—Prepare a sample solution by adding $20 \,\mu$ L of 0.2% sodium laurylsulfate TS to $20 \,\mu$ L of Teceleukin (Genetical Recombination). Perform the test as directed under Liquid Chromatography <2.01> using $20 \,\mu$ L of this solution under the following conditions. Determine using automated integration the teceleukin peak area, A_2 , and the peak area, A_1 , of the dimer with a relative retention time of 0.8 to 0.9 in relation to teceleukin. The amount of the dimer is not more than 1.0% by the following formula.

Amount (%) of dimer =
$$A_1/(A_1 + A_2) \times 100$$

Operating conditions—

Detector: Ultravioletabsorption photometer (wavelength: 220 nm).

Column: A stainless steel column with an inside diameter of 7.5 mm and 60 cm in length, packed with gycol etherifized silica gel for liquid chromatography (particle diameter: 10 μ m).

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

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 0.1 mol/L sodium phosphate buffer, pH 7.0, to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of teceleukin is between 30 and 40 minutes.

System suitability-

System performance: Add 20 μ L of 0.2% sodium lauryl sulfate TS to 20 μ L of a solution consisting of 5 mg of carbonic anhydrase and 5 mg of α -lactoalbumin dissolved in 100 mL of water. When 20 μ L of this solution is tested under the above conditions, carbonic anhydrase and α -lactoalbumin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: Measure exactly 1.0 mL of the sample solution, add the mobile phase to exactly 20 mL. To exactly 1 mL of this solution add the mobile phase to make exactly 10 mL. When the test is repeated 3 times with $20 \,\mu\text{L}$ of

this solution under the above conditions, the relative standard deviation of the teceleukin peak area is not more than 7%.

(5) Other related proteins—Perform the test on $5 \mu L$ of Teceleukin (Genetical Recombination) as directed under Liquid Chromatography $\langle 2.01 \rangle$ under the following conditions, and measure the area of each peak using automatic integration. When the amounts are determined by the area percent method, the total amount of peaks other than the teceleukin and solvent peaks is not more than 1.0%.

Operating conditions—

Detector: Ultravioletabsorption photometer (wavelength: 220 nm).

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

Column temperature: A constant temperature of about 30° C.

Mobile phase A: A solution of trifluoroacetic acid in a mixture of water and acetonitrile (19:1) (1 in 1000).

Mobile phase B: A solution of trifluoroacetic acid in acetonitrile (7 in 10,000).

Mobile phase flow: Control the concentration gradient by changing the mobile phase A and mobile phase B as shown in the table below.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
$0 \sim 12$	$60 \rightarrow 50$	$40 \rightarrow 50$
$12 \sim 25$	50	50
$25 \sim 45$	$50 \rightarrow 0$	$50 \rightarrow 100$
45 ~ 50	0	100

Flow rate: 1.0 mL/min.

Time span of measurement: a range that is approximately 1.2-fold the retention time of teceleukin.

System suitability-

System performance: Add 3.8 μ L of water and 16.6 μ L of polysorbet 80 solution (1 in 100) to 83.6 μ L of Teceleukin (Genetical Recombination) and let stand for at least one hour. When 5 μ L of this solution is tested by running under the above conditions, there is complete separation between the teceleukin peak and the peak with a relative retention time of about 0.98 in relation to the teceleukin peak.

(6) Acetic acid—Measure exactly 0.25 mL of Teceleukin (Genetical Recombination) and add exactly 0.25 mL of the internal standard solution to make the sample solution. Separately, measure exactly 3 mL of acetic acid (100) and add water to make exactly 100 mL. Take exactly 10 mL of this solution and add water to make exactly 100 mL. Measure exactly 2 mL of this solution and add exactly 2 mL of the internal standard solution to make the standard solution. Perform the test with 1 μ L each of the sample solution and the standard solutions. Calculate the ratios of the peak area of acetic acid to that of the internal standard, Q_T and Q_S , and the amount of acetic acid ($C_2H_4O_2$) in 1 mL of Teceleukin (Genetical Recombination) determined by the following formula is between 2.85 and 3.15 mg.

Amount (mg) of acetic acid $(C_2H_4O_2)$ in 1 mL of Teceleukin (Genetical Recombination)

 $= Q_{\rm T}/Q_{\rm S} \times 1.5 \times 1.049 \times 2$

1.5: Concentration (μ L/mL) of acetic acid (100) in the standard solution

1.049: Density $(mg/\mu L)$ of acetic acid (100) at 25°C 2: Dilution coefficient

Internal standard solution—Diluted propionic acid (1 in 500).

Operating conditions—

Detector: Hydrogen flame ionization detector.

Column: A glass column with an inside diameter of 1.2 mm and 40 m in length, whose inside is covered with chemically-bound polyethylene glycol for gas chromatography $1.0 \,\mu$ m in thickness.

Column temperature: A constant temperature of about 110°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of acetic acid is about 8 minutes.

System suitability—

System performance: When $1 \,\mu L$ of the standard solution is run under the above conditions, acetic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.0.

System repeatability: When the test is repeatedly run 6 times under the above conditions using $1 \,\mu\text{L}$ of standard solution, the relative standard deviation of the ratio of the acetic acid peak area to the internal standard peak area is not more than 5%.

Bacterial endotoxins <4.01> Less than 5EU per mg of protein.

Specific activity Accurately measure an appropriate amount of Teceleukin (Genetical Recombination), and add water accurately so that 1 mL contains about 0.1 mg. This is the sample solution. Separately, measure precisely about 25 mg of human serum albumin for assay, dissolve in water, and add water to make 50 mL. Measure exactly an appropriate amount of this solution, and accurately dilute with water to make standard solutions with concentrations of 0.05, 0.10, and 0.15 mg/mL. Accurately measure 1 mL each of the sample solution, the standard solutions, and water, add 2.5 mL of alkaline copper solution, mix, leave for at least 10 minutes to dissolve, add exactly 2.5 mL of water and 0.5 mL of diluted Folin reagent (1 in 2), immediately shake vigorously, and then leave for 30 minutes at 37°C. Perform the test on these solutions, with water as a control, as directd under Ultraviolet-visible Spectrophotometry <2.24>, and measure the absorbance at 750 nm. With the concentration of the standard solution as the x-axis and the absorbance as the y-axis, perform linear regression using their respective reciprocals, and calculate the protein content.

Calculate the ratio of the potency determined by Assay and the protein content.

Assay Accurately measure an appropriate amount of Teceleukin (Genetical Recombination) and, depending on the cell sensitivity, dilute precisely by adding culture medium for assay of teceleukin to a constant concentration of 10 to 50 units/mL (estimated value). This is the sample solution. Separately, dissolve Interleukin-2 Reference Substance in 1

mL of sterilized purified water, and, depending on the cell sensitivity, dilute precisely by adding culture medium for assay of teceleukin to a constant concentration of 10 to 50 units/mL. This is the standard solution. Add exactly $50 \,\mu\text{L}$ of culture medium for assay of teceleukin to all but 8 wells in a microtest plate. Add 50 μ L of the sample solution and the standard solution to 2 wells each containing culture medium for assay of teceleukin. From these 4 wells, remove exactly $50\,\mu\text{L}$ and add to 4 other wells containing culture medium for assay of teceleukin. From these 4 wells, remove exactly $50\,\mu\text{L}$ and add to 4 other wells containing culture medium for assay of teceleukin and repeat this procedure to prepare 2 wells that contain each of 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, and 1/256 dilutions of the sample and standard solutions. Add 50 μ L of the standard solution to each of the 8 empty wells to make maximum uptake controls. Eight wells containing only culture medium for assay of teceleukin serve as the minimum uptake controls. After adding exactly 50 μ L of cell suspension solution for teceleukin to each well in a microtest plate, leave for 15 to 17 hours in an incubator at 37°C filled with air containing 5% carbon dioxide. After adding 25 μ L of MTT TS to each of the wells in the plate, leave for 4 hours in an incubator at 37°C filled with air containing 5% carbon dioxide. Transfer the culture medium in all of the wells to empty wells in another microtest plate. To each of the empty wells from which the culture medium was removed, add $100 \,\mu\text{L}$ of hydrochloric acid-2-propanol TS, and then shake the plates horizontally for 5 minutes to elute the pigment. After returning the transferred culture medium to each original well, perform the test with the solution in each well, determine the difference in absorption at wavelengths of 560 nm and 690 nm, and calculate the mean values of the identical respective solutions in the two wells (dilution solutions of the sample solution and standard solutions) as well as the 8 wells containing the maximum or minimum uptake controls. Prepare standard curves by plotting the values obtained from each dilution solution of the sample solution, with the dilution coefficient of the sample solution on the microtest plates in logarithmic scale on the horizontal axis and the absorbance on the vertical axis. Determine the mean absorbance values of the maximum and minimum uptake controls, find the values on the standard curve, and then calculate the dilution coefficient, $D_{\rm T}$. Perform the same plot for the dilution solution of the standard solution, calculate the dilution coefficient, $D_{\rm S}$, and then calculate the potency in 1 mL by the following formula.

Teceleukin potency (units) in 1 mL of Teceleukin (Genetical Recombination) = $S \times D_T/D_S \times d$

S: Concentration of standard solution (units/mL) d: Dilution coefficient when sample solution prepared

Containers and storage Containers—Tight containers Storage—Store at -70°C or below.

Teceleukin for Injection (Genetical Recombination)

注射用テセロイキン(遺伝子組換え)

Teceleukin for Injection (Genetical Recombination) is a preparation for injection which is dissolved before use.

It contains not less than 70.0% and not more than 150.0% of the labeled amount of teceleukin (genetical recombination) ($C_{698}H_{1127}N_{179}O_{204}S_8$: 15547.01).

Method of preparation Prepare as directed under Injection, with Teceleukin (Genetical Recombination).

Description Teceleukin for Injection (Genetical Recombination) occurs as a white, light mass or powder.

Identification (1) Dissolve the content of 1 vial of Teceleukin for Injection (Genetical Recombination) in 1 mL of sterilized purified water, dilute exactly with culture medium for assay of teceleukin to make the sample stock solution containing about 200 units per mL (estimate). Proceed as directed in the Identification (1) under Teceleukin (Genetical Recombination).

(2) Dissolve 0.242 g of 2-amino-2-hydroxymethyl-1,3propanediol, 5.0 g of sodium lauryl sulfate and 74 mg of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 60 mL of water. Adjust to pH 8.0 with 1 mol/L hydrochloric acid TS, add water to make 100 mL, and use this solution as the buffer solution for molecular mass determination. Separately, dissolve the content of 1 vial of Teceleukin for Injection (Genetical Recombination) in exactly 1 mL of water. To exactly 100 μ L of this solution add exactly 100 μ L of the buffer solution for molecular mass determination and $10 \,\mu L$ of 2-mercaptoethanol, and heat on a water bath for 5 minutes without allowing any water evaporation from the mixture. After cooling, add exactly $1 \mu L$ of bromophenol blue solution (1 in 2000), mix, and use this solution as the sample solution. Proceed as directed in Identification (3) under Teceleukin (Genetical Recombination): a band appears in the range of molecular mass between 14,000 and 16,000.

pH $\langle 2.54 \rangle$ Dissolve the content of one vial of Teceleukin for Injection (Genetical Recombination) in 1 mL of water: the pH of the solution is between 7.0 and 7.7.

Purity Clarity and color of solution—Dissolve the content of one vial of Teceleukin for Injection (Genetical Recombination) in 1 mL of water: the solution is clear and colorless.

Loss on drying Transfer the content of the vial of Teceleukin for Injection (Genetical Recombination) to a weighing bottle under the atmosphere not exceeding 10% relative humidity, and perform the test as directed in the Water content determination described in the Minimum Requirements for Biological Products: not more than 5%.

Bacterial endotoxins <4.01> Less than 5 EU/350,000 units.

Uniformity of dosage units $\langle 6.02 \rangle$ It meets the requirement of the Mass variation test. Calculate as |M - A| = 0.

Foreign insoluble matter <6.06> Perform the test according

to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

1465

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Dissolve the content of 1 vial of Teceleukin for Injection (Genetical Recombination) in exactly 1 mL of sterilized purified water, dilute exactly with culture medium for assay of teceleukin to make the sample solution containing a definite concentration of 10 to 50 units/mL (estimate). Proceed as directed in the Assay under Teceleukin (Genetical Recombination), and calculate the amount (unit) of teceleukin in 1 vial by the following formula.

Amount (unit) of teceleukin in 1 vial = $S \times D_T / D_S \times d \times 1$

- S: Concentration of the standard solution (unit/mL)
- d: Dilution coefficient when sample solution prepared
- 1: Volume (mL) of the sample solution

Containers and storage Containers—Hermetic containers. Storage—Light-resistant, not exceeding 10°C, avoiding freezing.

Tegafur

テガフール



C₈H₉FN₂O₃: 200.17

5-Fluoro-1-[(2*RS*)-tetrahydrofuran-2-yl]uracil [*17902-23-7*]

Tegafur, when dried, contains not less than 98.0% of $C_8H_9FN_2O_3$.

Description Tegafur occurs as a white, crystalline powder. It is soluble in methanol and in acetone, and sparingly soluble in water and in ethanol (95).

It dissolves in dilute sodium hydroxide TS.

A solution of Tegafur in methanol (1 in 50) shows no optical rotation.

Identification (1) Prepare the test solution with 0.01 g of Tegafur as directed under Oxygen Flask Combustion Method $\langle 1.06 \rangle$, 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 $\langle 1.09 \rangle$ (2) for fluoride.

(2) Determine the absorption spectrum of a solution of Tegafur in 0.01 mol/L sodium hydroxide TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Tegafur, previously dried, as directed in the potassium bro-

mide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize the sample with a mixture of methanol and acetone (1:1), filter and dry the crystals, and perform the test with the crystals.

pH <2.54> Dissolve 0.5 g of Tegafur in 50 mL of water: the pH of this solution is between 4.2 and 5.2.

Melting point <2.60> 166 – 171°C

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 $\langle 1.03 \rangle$ —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 using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(3) Heavy metals <1.07>—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 using this solution as the test solution. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic $\langle 1.11 \rangle$ —Prepare the test solution in a platinum crucible with 1.0 g of Tegafur according to Method 4, incinerating by ignition between 750°C and 850°C, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Tegafur in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample 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 $\langle 2.03 \rangle$. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with 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 sample solution are not more intense than the spot from the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 $\langle 2.50 \rangle$ the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS). Perform a blank determination.

Each mL of $\frac{1}{60}$ mol/L potassium bromate VS = 10.01 mg of C₈H₉FN₂O₃ Containers and storage Containers-Tight containers.

Teicoplanin

テイコプラニン



Teicoplanin A2-1

C88H95Cl2N9O33: 1877.64

(3S, 15R, 18R, 34R, 35S, 38S, 48R, 50aR) - 34 - (2-Acetylamino-2-deoxy- β -D-glucopyranosyloxy)-15-amino-22, 31-dichloro-56-[2-(4Z)-dec-4-enoylamino-2-deoxy- β -D-glucopyranosyloxy]-6, 11, 40, 44-tetrahydroxy-42-(α -Dmannopyranosyloxy)-2, 16, 36, 50, 51, 59-hexaoxo-2, 3, 16, 17, 18, 19, 35, 36, 37, 38, 48, 49, 50, 50atetradecahydro-1H, 15H, 34H-20, 23: 30, 33dietheno-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 A₂₋₂

C₈₈H₉₇Cl₂N₉O₃₃: 1879.66

(3S,15R,18R,34R,35S,38S,48R,50aR)-34-(2-

Acetylamino-2-deoxy- β -D-glucopyranosyloxy)-15-amino-22,31-dichloro-56-[2-deoxy-2-(8-methylnonanoylamino)- β -D-glucopyranosyloxy]-6,11,40,44-tetrahydroxy-42-(α -D-mannopyranosyloxy)-2,16,36,50,51,59-hexaoxo-2,3,16,17,18,19,35,36,37,38,48,49,50,50a-tetradecahydro-

JP XVI

1*H*,15*H*,34*H*-20,23:30,33-dietheno-3,18:35,48bis(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 A₂₋₃

C88H97Cl2N9O33: 1879.66

(3S,15R,18R,34R,35S,38S,48R,50aR)-34-(2-Acetylamino-2-deoxy- β -D-glucopyranosyloxy)-15-amino-22,31-dichloro-56-(2-decanoylamino-2-deoxy- β -D-glucopyranosyloxy)-6,11,40,44-tetrahydroxy-42-(α -D-mannopyranosyloxy)-2,16,36,50,51,59-hexaoxo-

2,3,16,17,18,19,35,36,37,38,48,49,50,50a-tetradecahydro-1*H*,15*H*,34*H*-20,23:30,33-dietheno-3,18:35,48bis(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 A₂₋₄

C89H99Cl2N9O33: 1893.68

 $(3S,15R,18R,34R,35S,38S,48R,50aR)-34-(2-Acetylamino-2-deoxy-β-D-glucopyranosyloxy)-15-amino-22,31-dichloro-56-[2-deoxy-2-(8-methyldecanoylamino)-β-D-glucopyranosyloxy]-6,11,40,44-tetrahydroxy-42-(α-D-mannopyranosyloxy)-2,16,36,50,51,59-hexaoxo-2,3,16,17,18,19,35,36,37,38,48,49,50,50a-tetradecahydro-1H,15H,34H-20,23:30,33-dietheno-3,18:35,48-bis(iminomethano)-4,8:10,14:25,28:43,47-tetrametheno-28H-[1,14,6,22]dioxadiazacyclooctacosino[4,5-m][10,2,16]-benzoxadiazacyclotetracosine-38-carboxylic acid [91032-37-0]$

Teicoplanin A₂₋₅

 $C_{89}H_{99}Cl_2N_9O_{33}$: 1893.68

 $(3S,15R,18R,34R,35S,38S,48R,50aR)-34-(2-Acetylamino-2-deoxy-β-D-glucopyranosyloxy)-15-amino-22,31-dichloro-56-[2-deoxy-2-(9-methyldecanoylamino)-β-D-glucopyranosyloxy]-6,11,40,44-tetrahydroxy-42-(α-D-mannopyranosyloxy)-2,16,36,50,51,59-hexaoxo-2,3,16,17,18,19,35,36,37,38,48,49,50,50a-tetradecahydro-1H,15H,34H-20,23:30,33-dietheno-3,18:35,48-bis(iminomethano)-4,8:10,14:25,28:43,47-tetrametheno-28H-[1,14,6,22]dioxadiazacyclooctacosino[4,5-m][10,2,16]-benzoxadiazacyclotetracosine-38-carboxylic acid [91032-38-1]$

Teicoplanin A_{3-1} $C_{72}H_{68}Cl_2N_8O_{28}$: 1564.25 (3S,15R,18R,34R,35S,38S,48R,50aR)-34-(2-Acetylamino-2-deoxy- β -D-glucopyranosyloxy)-15-amino-22,31-dichloro-6,11,40,44,56-pentahydroxy-42-(α -Dmannopyranosyloxy)-2,16,36,50,51,59-hexaoxo-2,3,16,17,18,19,35,36,37,38,48,49,50,50a-tetradecahydro-1H,15H,34H-20,23:30,33-dietheno-3,18:35,48bis(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 [93616-27-4]

[61036-62-2, Teicoplanin]

Teicoplanin is a mixture of glycopeptide substances having antibacterial activity produced by the growth of *Actinoplanes teichomyceticus*.

It contains not less than $900 \ \mu g$ (potency) and not more than $1120 \ \mu g$ (potency) per 1 mg, calculated on the anhydrous, de-sodium chloride and de-residual solvents basis. The potency of Teicoplanin is expressed as mass (potency) of teicoplanin (C₇₂₋₈₉H₆₈₋₉₉Cl₂N₈₋₉O₂₈₋₃₃).

Description Teicoplanin occurs as a white to light yellowish white powder.

It is freely soluble in water, sparingly soluble in N,Ndimethylformamide, and practically insoluble in acetonitrile, in methanol, in ethanol (95), in acetone, in acetic acid (100) and in diethyl 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 absorption spectra of Teicoplanin and Teicoplanin RS as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the spectrum of Teicoplanin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH $\langle 2.54 \rangle$ 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 of the active principle Dissolve about 20 mg of Teicoplanin in water to make 10 mL, and use this solution as the sample solution. Perform the test with $20 \,\mu$ L of the sample solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and calculate the sum of peak areas of teicoplanin A₂ group, S_a, the sum of peak areas of teicoplanin A₃ group, S_b, and the sum of peak areas of other contents, S_c from the sample solution by the automatic integration method. Calculate the content ratio of them by the formula given below: teicoplanin A₂ group, teicoplanin A₃ group, and the other are not less than 80.0%, not more than 15.0% and not more than 5.0%, respectively.

The elution order of each content and the relative retention time of each content to the retention time of teicoplanin $A_{2,2}$ are shown in the following table.

Name of content	Elution order	Relative retention time
teicoplanin A ₃ group		≦0.42
teicoplanin A ₃₋₁	1	0.29
teicoplanin A ₂ group		$0.42 < , \le 1.25$
teicoplanin A ₂₋₁	2	0.91
teicoplanin A ₂₋₂	3	1.00
teicoplanin A ₂₋₃	4	1.04
teicoplanin A_{2-4}	5	1.17
teicoplanin A_{2-5}	6	1.20
others		1.25 <

Content ratio (%) of teicoplanin A₂ group = $S_a/(S_a + 0.83S_b + S_c) \times 100$

Content ratio (%) of teicoplanin A₃ group
=
$$0.83S_b/(S_a + 0.83S_b + S_c) \times 100$$

Content ratio (%) of others = $S_c/(S_a + 0.83S_b + S_c) \times 100$

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

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

Mobile phase A: Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in 1650 mL of water, add 300 mL of acetonitrile, adjust 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.

Flowing of the mobile phase: Flow mobile phase A for 10 minutes before injection. After injection, control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 - 32 32 - 40 40 - 42	$\begin{array}{rrr} 100 \rightarrow & 70 \\ 70 \rightarrow & 50 \\ 50 \rightarrow & 100 \end{array}$	$\begin{array}{c} 0 \rightarrow 30 \\ 30 \rightarrow 50 \\ 50 \rightarrow 0 \end{array}$

Flow rate: 1.8 mL per minute.

Time span of measurement: About 1.7 times as long as the retention time of teicoplanin A_{2-2} , beginning after the solvent peak.

System suitability—

Test for required detection: Confirm that peak height of teicoplanin A_{2-2} obtained from the sample solution is equivalent to 90% of the full scale.

System performance: When the procedure is run with 20 μ L of the sample solution under the above operating conditions, the symmetry factor of the peak of teicoplanin A₃₋₁ is not more than 2.2.

System repeatability: When the test is repeated 3 times with $20 \,\mu\text{L}$ of the sample solution under the above operating conditions, the relative standard deviation of the peak area of teicoplanin A₂₋₂ is not more than 2.0%.

Purity (1) Clarity and color of solution—Being specified separately.

(2) Sodium chloride—Weigh accurately about 0.5 g of Teicoplanin, dissolve in 50 mL of water, titrate $\langle 2.50 \rangle$ with 0.1 mol/L silver nitrate VS (indicator: 1 mL of potassium chromate TS), and calculate an amount of sodium chloride: not more than 5.0%.

Each mL of 0.1 mol/L silver nitrate VS = 5.844 mg of NaCl

- (3) Heavy metals <1.07>—Being specified separately.
- (4) Arsenic <1.11>—Being specified separately.

(5) Residual solvents <2.46>—Weigh accurately about 0.1 g of Teicoplanin, dissolve in N, N-dimethylformamide to make exactly 10 mL, and use this solution as the sample 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,Ndimethylformamide to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly $4\,\mu\text{L}$ each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions. Determine the peak area of methanol, A_1 , and the peak area of acetone, A_2 , obtained from the sample solution, and the peak area of methanol, A_{S1} , and the peak area of acetone, A_{S2} , obtained from the standard solution by the automatic integration method, and calculate the amounts of methanol and acetone by the following formula: not more than 0.5% and not more than 1.0%, respectively.

Amount (%) of methanol
=
$$M_{S1} \times A_1/A_{S1} \times 0.001 \times 1/M_T \times 100$$

Amount (%) of acetone
= $M_{S2} \times A_2/A_{S2} \times 0.001 \times 1/M_T \times 100$

 M_{S1} : Amount (g) of methanol M_{S2} : Amount (g) of acetone M_T : Amount (g) of Teicoplanin

Operating conditions—

Detector: Hydrogen flame-ionization detector.

Column: A glass column 2 mm in inside diameter and 3 m in length, packed with graphite carbon for gas chromatography, 150 to 180 μ m in particle diameter, coated with 0.1% of polyethylene glycol esterified.

Column temperature: Inject the sample at a constant temperature of about 70°C, maintain the temperature for 4 minutes, then program to increase the temperature at the rate of 8° C per minute to 210° C.

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 5 minutes, respectively.

System suitability-

Test for required detection: Confirm that the peak height of acetone obtained from $4 \mu L$ of the standard solution is equivalent to about the full scale.

System performance: When the procedure is run with $4 \mu L$ 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 $4 \mu L$ of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of acetone is not more than 3%.

Water $\langle 2.48 \rangle$ Not more than 15.0% (0.2 g, volumetric titration, direct titration).

Bacterial endotoxins <4.01> Less than 0.75 EU/mg (potency).
Blood pressure depressant Being specified separately.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism-Bacillus subtilis ATCC 6633

(ii) Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Teicoplanin 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 at not exceeding 5°C and use within 14 days. Take exactly a suitable amount of this solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 160 μ g (potency) and 40 μ g (potency), and use these solutions as the high concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Teicoplanin equivalent to about 50 mg (potency), dissolve in phosphate buffer solution, pH 6.0 to make exactly 50 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 160 μ g (potency) and 40 μ g (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers. Storage—Light-resistant, and not exceeding 5° C.

Temocapril Hydrochloride

テモカプリル塩酸塩



C₂₃H₂₈N₂O₅S₂.HCl: 513.07

2-[(2*S*,6*R*)-6-{[(1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino}-5-oxo-2-(thiophen-2-yl)-2,3,6,7-tetrahydro-1,4-thiazepin-4(5*H*)-yl] acetic acid monohydrochloride [*110221-44-8*]

Temocapril Hydrochloride contains not less than 99.0% and not more than 101.0% of $C_{23}H_{28}N_2O_5S_2$. HCl, calculated on the anhydrous basis.

Description Temocapril Hydrochloride occurs as a white crystalline powder.

It is freely soluble in ethanol (99.5), and vey slightly soluble in water.

Identification (1) Determine the absorption spectrum of a solution of Temocapril Hydrochloride in ethanol (99.5) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of

Temocapril Hydrochloride as directed in the paste method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Temocapril Hydrochloride in ethanol (99.5) (1 in 100) responds to the Qualitative Tests <1.09> (2) for chloride.

Optical rotation $\langle 2.49 \rangle$ [α]_D²⁰: +60 - +64° (0.2 g, calculated on the anhydrous basis, ethanol (99.5), 20 mL, 100 mm).

Purity (1) Heavy metals $\langle 1.07 \rangle$ —Proceed with 1.0 g of Temocapril 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—Dissolve 50 mg of Temocapril Hydrochloride in 100 mL of diluted acetonitrile (1 in 2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted acetonitrile (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly $10 \,\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak other than temocapril obtained from the sample solution is not larger than 1/5 times the peak area of temocapril from the standard solution, and the total area of the peaks other than temocapril from the sample solution is not larger than 1/2 times the peak area of temocapril from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 234 nm).

Column: A stainless steel column 6.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

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

Mobile phase: A mixture of diluted phosphoric acid (1 in 500) and acetonitrile (63:37).

Flow rate: Adjust the flow rate so that the retention time of temocapril is about 11 minutes.

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

System suitability-

Test for required detectability: Pipet 1 mL of the standard solution, and add diluted acetonitrile (1 in 2) to make exactly 10 mL. Confirm that the peak area of temocapril obtained with 10 μ L of this solution is equivalent to 7 to 13% of that with 10 μ L of the standard solution.

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of temocapril are not less than 7000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of temocapril is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Water $\langle 2.48 \rangle$ Not more than 1.0% (0.3 g, coulometric titration).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.8 g of Temocapril Hydrochloride, dissolve in 80 mL of a mixture of acetic anhydrate and acetic acid (100) (7:3), and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

> Each mL of 0.1 mol/L perchloric acid VS = $51.31 \text{ mg of } C_{23}H_{28}N_2O_5S_2.HCl$

Containers and storage Containers-Well-closed containers.

Temocapril Hydrochloride Tablets

テモカプリル塩酸塩錠

Temocapril Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of temocapril hydrochloride ($C_{23}H_{28}N_2O_5S_2$. HCl: 513.07).

Method of preparation Prepare as directed under Tablets, with Temocapril Hydrochloride.

Identification To an amount of powdered Temocapril Hydrochloride Tablets, equivalent to 2.5 mg of Temocapril Hydrochloride according to the labeled amount, add 25 mL of diluted acetonitrile (1 in 2), shake vigorously for 10 minutes, and centrifuge. To 5 mL of the supernatant liquid add diluted acetonitrile (1 in 2) to make 25 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: it exhibits a maximum between 232 nm and 236 nm.

Uniformity of dosage units $\langle 6.02 \rangle$ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Temocapril Hydrochloride Tablets add exactly 20 mL of diluted acetonitrile (1 in 2), and agitate for 10 minutes with the aid of ultrasonic waves. Furthermore, shake for 10 minutes, and centrifuge. Pipet VmL of the supernatant liquid equivalent to about 0.8 mg of temocapril hydrochloride (C₂₃H₂₈N₂O₅S₂.HCl), add exactly 2 mL of the internal standard solution, then add diluted acetonitrile (1 in 2) to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of temocapril hydrochloride for assay (separately determine the water <2.48> in the same manner as Temocapril Hydrochloride), dissolve in diluted acetonitrile (1 in 2) to make exactly 200 mL. Pipet 4 mL of this solution, add exactly 2 mL of the internal standard solution, then add diluted acetonitrile (1 in 2) to make 20 mL, and use this solution as the standard solution. Perform the test with exactly $10 \,\mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of temocapril to that of the internal standard.

> Amount (mg) of temocapril hydrochloride ($C_{23}H_{28}N_2O_5S_2$.HCl) = $M_S \times Q_T/Q_S \times 1/V \times 2/5$

 $M_{\rm S}$: Amount (mg) of temocapril hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of propyl parahydroxybenzoate in diluted acetonitrile (1 in 2) (1 in 3000). Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability-

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, temocapril and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of temocapril to that of the internal standard is not more than 1.0%.

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Temocapril Hydrochloride Tablets is not less than 85%.

Start the test with 1 tablet of Temocapril Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding $0.45 \,\mu\text{m}$. 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 $1.1 \,\mu g$ of temocapril hydrochloride (C23H28N2O5S2.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of temocapril hydrochloride for assay (separately determine the water <2.48> in the same manner as Temocapril Hydrochloride), and dissolve in diluted acetonitrile (1 in 2) to make exactly 50 mL. Pipet 5 mL of this 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 exactly 50 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak area, $A_{\rm T}$ and $A_{\rm S}$, of temocapril of both solutions.

Dissolution rate (%) with respect to the labeled amount of temocapril hydrochloride ($C_{23}H_{28}N_2O_5S_2$.HCl)

$$= M_{\rm S} \times A_{\rm T}/A_{\rm S} \times V'/V \times 1/C \times 9/2$$

- $M_{\rm S}$: Amount (mg) of temocapril hydrochloride for assay, calculated on the anhydrous basis
- C: Labeled amount (mg) of temocapril hydrochloride (C₂₃H₂₈N₂O₅S₂.HCl) in 1 tablet

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: A mixture of diluted phosphoric acid (1 in 500) and acetonitrile (43:32).

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

System suitability-

System performance: When the procedure is run with 50 μ L of the standard solution under the above operating con-

ditions, the number of theoretical plates and the symmetry factor of the peak of temocapril are not less than 9000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with $50 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of temocapril is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Temocapril Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of temocapril hydrochloride (C23H28N2O5S2.HCl), add exactly 20 mL of the internal standard solution, and agitate for 10 minutes with the aid of ultrasonic waves. Furthermore, shake for 10 minutes, and centrifuge. To 2 mL of the supernatant liquid add diluted acetonitrile (1 in 2) to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of temocapril hydrochloride for assay (separately determine the water $\langle 2.48 \rangle$ in the same manner as Temocapril Hydrochloride), and dissolve in diluted acetonitrile (1 in 2) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution, then add diluted acetonitrile (1 in 2) to make 100 mL, and use this solution as the standard solution. Perform the test with $10 \,\mu L$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of temocapril to that of the internal standard.

> Amount (mg) of temocapril hydrochloride ($C_{23}H_{28}N_2O_5S_2$.HCl) = $M_S \times Q_T/Q_S \times 1/5$

 $M_{\rm S}$: Amount (mg) of temocapril hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of propyl parahydroxybenzoate in diluted acetonitrile (1 in 2) (1 in 3000). *Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 234 nm).

Column: A stainless steel column 6.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

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

Mobile phase: A mixture of diluted phosphoric acid (1 in 500) and acetonitrile (63:37).

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

System suitability-

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, temocapril and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of temocapril to that of the internal standard is not more than 1.0%.

Containers and storage Containers-Well-closed containers.

Teprenone



C₂₃H₃₈O: 330.55 (5*E*,9*E*,13*E*)-6,10,14,18-Tetramethylnonadeca-5,9,13,17-tetraen-2-one (5*Z*,9*E*,13*E*)-6,10,14,18-Tetramethylnonadeca-5,9,13,17-tetraen-2-one [6809-52-5]

Teprenone contains not less than 97.0% and not more than 101.0% of $C_{23}H_{38}O$.

Teprenone is comprised of mono-cis and all-trans isomers, with their ratio being about 2:3.

Description Teprenone occurs as a colorless to slightly yellowish clear oily liquid, with slight, characteristic odor.

It is miscible with ethanol (99.5), with ethyl acetate and with hexane.

It is practically insoluble in water.

It is oxidized by air, and gradually turns yellow.

Identification (1) To 2 mL of a solution of Teprenone in ethanol (99.5) (1 in 100) add 1 mL of a solution of phosphomolybdic acid *n*-hydrate in acetic acid (100) (1 in 100), heat in a water bath for 5 minutes, and continue heating with addition of 5 to 6 drops of sulfuric acid: blue to bluish green color develops.

(2) To 2 mL of a solution of Teprenone in ethanol (99.5) (1 in 100) add 2 mL of 2,4-dinitrophenylhydrazine TS, and shake: a yellow to orange-yellow precipitate is formed.

(3) Determine the infrared absorption spectrum of Teprenone as directed in the liquid film method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Teprenone RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Refractive index <2.45> $n_{\rm D}^{20}$: 1.485 – 1.491

Specific gravity <2.56> d_{20}^{20} : 0.882 - 0.890

Purity (1) Clarity and color of solution—To 1.0 mL of Teprenone add 9 mL of ethanol (99.5) and shake: the solution is clear, and its absorbance at 400 nm determined as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$ is not more than 0.02.

(2) Heavy metals $\langle 1.07 \rangle$ —Proceed with 1.0 g of Teprenone 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 30 mg of Teprenone in 6 mL of hexane, and use this solution as the sample solution. Perform the test with 3 μ L of the sample solution as directed under Gas Chromatography <2.02> according to the following conditions. Determine each peak area from the sample

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solution by the automatic integration method and calculate the amounts of them by the area percentage method: the peak area of the di-cis isomer of teprenone, having the relative retention time of about 0.8 with respect to the all-trans isomer of teprenone, is not more than 0.5%, and each area of the peaks for the mono-cis and all-trans isomers of the teprenone and for those other than mentioned above is not more than 0.2%. Furthermore, the total area of the peaks other than the mono-cis, all-trans and di-cis isomers of teprenone is not more than 1.0%.

Operating conditions—

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

Time span of measurement: About 2 times as long as the retention time for the all-trans isomer of teprenone beginning after the solvent peak.

System suitability-

Test for required detectability: To 1 mL of the sample solution add hexane to make 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 hexane to make exactly 10 mL. Confirm that the sum of the peak areas of the mono-cis and all-trans isomers of teprenone obtained from 3 μ L of this solution is 7 to 13% of the peak areas of the mono-cis and all-trans isomers of teprenone from 3 μ L of the solution for system suitability test.

System performance: When the procedure is run with $3 \mu L$ of the solution for system suitability test under the above operating conditions, the mono-cis and all-trans isomers of teprenone are eluted in this order with the resolution between these peaks being not less than 1.1.

System repeatability: When the test is repeated 6 times with $3 \mu L$ of the solution for system suitability test under the above operating conditions, the relative standard deviation of the sum of the peak areas of the mono-cis and all-trans isomers of teprenone is not more than 3.0%.

(4) Residual solvent—Being specified separately.

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Isomer ratio Dissolve 30 mg of Teprenone in 6 mL of hexane, and use this solution as the sample solution. Perform the test with 3μ L of the sample solution as directed under Gas Chromatography $\langle 2.02 \rangle$ according to the following conditions. Determine the areas of two adjacent peaks, A_a and A_b , having retention times of about 18 minutes, where A_a is the peak of the mono-cis isomer, having the shorter retention time, and A_b is the peak area of the all-trans isomer, having the longer retention time: A_a/A_b is 0.60 to 0.70.

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability-

Proceed as directed in the system suitability in the Purity (3).

Assay Weigh accurately about 50 mg each of Teprenone and Teprenone RS, dissolve each in exactly 5 mL of the internal standard solution, add ethyl acetate to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 3 μ L each of the sample solution and standard solution as directed under Gas Chromatography $\langle 2.02 \rangle$ according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of teprenone (sum of the peak areas of mono-cis and alltrans isomers) to that of the internal standard.

Amount (mg) of teprenone (C₂₃H₃₈O) = $M_{\rm S} \times Q_{\rm T}/Q_{\rm S}$

 $M_{\rm S}$: Amount (mg) of Teprenone RS

Internal standard solution—A solution of di-n-butyl phthalate in ethyl acetate (1 in 200).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 4 mm in inside diameter and 2 m in length, packed with 149 to $177 \,\mu$ m siliceous earth for gas chromatography coated in 5% with polyethylene glycol 2-nitroterephthalate.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of the peak of the all-trans isomer of teprenone, the larger of the two main peaks, having the retention time about 18 minutes, becomes 19 minutes.

System suitability—

System performance: When the procedure is run with 3 μ L of the standard solution under the above operating conditions, the internal standard and the mono-cis and all-trans isomers of teprenone are eluted in this order with the resolution between the mono-cis and all-trans isomers being not less than 1.1.

System repeatability: When the test is repeated 6 times with $3 \mu L$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the sum of the peak areas of the mono-cis and all-trans isomers of teprenone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight Containers. Storage—Under Nitrogen atmosphere at 2 to 8°C.

Terbinafine Hydrochloride



C₂₁H₂₅N.HCl: 327.89

(2*E*)-*N*,6,6-Trimethyl-*N*-(naphthalen-1-ylmethyl)hept-2-en-4-yn-1-amine monohydrochloride [78628-80-5]

• HCI

Terbinafine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of $C_{21}H_{25}N.HCl.$

Description Terbinafine Hydrochloride occurs as a white to pale yellowish white crystalline powder.

It is freely soluble in methanol, in ethanol (99.5) and in acetic acid (100), and slightly soluble in water.

The pH of a solution of 1.0 g of Terbinafine Hydrochlo-

ride in 1000 mL of water is 3.5 to 4.5.

Melting point: about 205°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Terbinafine Hydrochloride in methanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Terbinafine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Terbinafine Hydrochloride in ethanol (99.5) (1 in 100) responds to the Qualitative Tests $\langle 1.09 \rangle$ (2) for chloride.

Purity (1) Heavy metals $\langle 1.07 \rangle$ —Proceed with 1.0 g of Terbinafine 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—Conduct this procedure using light-resistant vessels. Dissolve 50 mg of Terbinafine Hydrochloride in 100 mL of a mixture of water and acetonitrile (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of water and acetonitrile (1:1) to make exactly 100 mL. Pipet 5 mL of this 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 exactly $20 \,\mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of a dimer, having the relative retention time of about 1.7 with respect to terbinafine obtained from the sample solution is not larger than 1/2times the peak area of terbinafine from the standard solution, the area of the peak other than terbinafine and the dimer from the sample solution is not larger than the peak area of terbinafine from the standard solution, and the total area of the peaks other than terbinafine is not larger than 3 times the peak area of terbinafine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 3 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

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

Mobile phase A: To 700 mL of a mixture of methanol and acetonitrile (3:2) add 300 mL of a solution of triethylamine (1 in 500) adjusted to pH 7.5 with dilute acetic acid.

Mobile phase B: To 950 mL of a mixture of methanol and acetonitrile (3:2) add 50 mL of a solution of triethylamine (1 in 500) adjusted to pH 7.5 with dilute acetic acid.

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

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 - 4	100	0
4 - 25	$100 \rightarrow 0$	$0 \rightarrow 100$
25 - 30	0	100

Flow rate: Adjust the flow rate so that the retention time of terbinafine is about 15 minutes.

Time span of measurement: About 2 times as long as the retention time of terbinafine, beginning after the solvent peak.

System suitability-

Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of water and acetonitrile (1:1) to make exactly 20 mL. Confirm that the peak area of terbinafine obtained with 20 μ L of this solution is equivalent to 18 to 32% of that with 20 μ L of the standard solution.

System performance: Dissolve 20 mg of Terbinafine Hydrochloride in 20 mL of a mixture of water and acetonitrile (1:1), and irradiate under a short-wave lamp (main wavelength: 254 nm) for 1 hour. When the procedure is run with 20 μ L of this solution under the above operating conditions, the resolution between the peak of cis-terbinafine, having the relative retention time of about 0.94 with respect to terbinafine, and the peak of terbinafine is not less than 2.0.

System repeatability: When the test is repeated 6 times with $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of terbinafine is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.26 g of Terbinafine Hydrochloride, previously dried, dissolve in 5 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = $32.79 \text{ mg of } C_{21}H_{25}N.HCl$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Terbinafine Hydrochloride Cream

テルビナフィン塩酸塩クリーム

Terbinafine Hydrochloride Cream contains not less than 95.0% and not more than 105.0% of the labeled amount of terbinafine hydrochloride ($C_{21}H_{25}N.HCl$: 327.89).

Method of preparation Prepare as directed under Creams, with Terbinafine Hydrochloride.

Identification To quantity of Terbinafine Hydrochloride Cream, equivalent to 10 mg of Terbinafine Hydrochloride,

according to the labeled amount, dissolve in 20 mL of 2propanol, and use this solution as the sample solution. Separately, dissolve 10 mg of terbinafine hydrochloride for assay in 20 mL of 2-propanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with the upper layer of a mixture of 80 volumes of hexane, 20 volumes of ethyl acetate and 1 volume of ammonia solution (28) to a distance of about 15 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution shows the same *R*f value with the spot from the standard solution.

Assay Weigh accurately an amount of Terbinafine Hydrochloride Cream, equivalent to about 10 mg of terbinafine hydrochloride (C₂₁H₂₅N.HCl), dissolve in 2-propanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of terbinafine hydrochloride for assay, previously dried at 105 °C for 4 hours, and dissolve in 2-propanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the terbinafine peak areas, $A_{\rm T}$ and $A_{\rm S}$, of both solutions.

Amount (mg) of terbinafine hydrochloride (C₂₁H₂₅N.HCl) = $M_{\rm S} \times A_{\rm T}/A_{\rm S} \times 1/4$

 $M_{\rm S}$: Amount (mg) of terbinafine hydrochloride for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 282 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 125 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

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

Mobile phase: A mixture of a solution of tetramethylammonium hydroxide (9 in 2000) adjusted to pH 8.0 with diluted phosphoric acid (1 in 25), acetonitrile and tetrahydrofuran (2:2:1).

Flow rate: Adjust the flow rate so that the retention time of terbinafine is about 8.5 minutes.

System suitability-

System performance: Dissolve 40 mg of terbinafine hydrochloride for assay and 3.5 mg of terphenyl in 200 mL of methanol. When the procedure is run with 10 μ L of this solution under the above operating conditions, terphenyl and terbinafine 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 $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of terbinafine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Terbinafine Hydrochloride Solution

テルビナフィン塩酸塩液

Terbinafine Hydrochloride Solution is a liquid for external use.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of terbinafine hydrochloride ($C_{21}H_{25}N.HCl:$ 327.89).

Method of preparation Prepare as directed under Liquids and Solutions for Cutaneous Application, with Terbinafine Hydrochloride.

Identification To a volume of Terbinafine Hydrochloride Solution, equivalent to 10 mg of Terbinafine Hydrochloride according to the labeled amount, add methanol to make 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of terbinafine hydrochloride for assay in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with the upper layer of a mixture of 80 volumes of hexane, 20 volumes of ethyl acetate and 1 volume of ammonia solution (28) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution shows the same Rf value with the spot from the standard solution.

pH Being specified separately.

Assay Weigh accurately an amount of Terbinafine Hydrochloride Solution, equivalent to about 10 mg of terbinafine hydrochloride (C₂₁H₂₅N.HCl), add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of terbinafine hydrochloride for assay, previously dried at 105°C for 4 hours, dissolve in methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the terbinafine peak areas, $A_{\rm T}$ and $A_{\rm S}$, of both solutions.

Amount (mg) of terbinafine hydrochloride (C₂₁H₂₅N.HCl) = $M_{\rm S} \times A_{\rm T}/A_{\rm S} \times 1/4$

 $M_{\rm S}$: Amount (mg) of terbinafine hydrochloride for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 282 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 125 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

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

Mobile phase: A mixture of a solution of tetramethylammonium hydroxide (9 in 2000) adjusted to pH 8.0 with diluted phosphoric acid (1 in 25), acetonitrile and tetrahydrofuran (2:2:1).

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Flow rate: Adjust the flow rate so that the retention time of terbinafine is about 8.5 minutes.

System suitability-

System performance: Dissolve 40 mg of terbinafine hydrochloride for assay and 3.5 mg of terphenyl in 200 mL of methanol. When the procedure is run with 10 μ L of this solution under the above operating conditions, terphenyl and terbinafine 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 $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of terbinafine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Terbinafine Hydrochloride Spray

テルビナフィン塩酸塩スプレー

Terbinafine Hydrochloride Spray contains not less than 95.0% and not more than 105.0% of the labeled amount of terbinafine hydrochloride ($C_{21}H_{25}N.HCl$: 327.89).

Method of preparation Prepare as directed under Pump Sprays for Cutaneous Application, with Terbinafine Hydrochloride.

Identification To an amount of Terbinafine Hydrochloride Spray, equivalent to 10 mg of Terbinafine Hydrochloride, add methanol to make 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of terbinafine hydrochloride for assay in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with the upper layer of a mixture of 80 volumes of hexane, 20 volumes of ethyl acetate and 1 volume of ammonia solution (28) to a distance of about 15 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution shows the same Rf value with the spot from the standard solution.

pH Being specified separately.

Assay Weigh accurately an amount of Terbinafine Hydrochloride Spray, equivalent to about 10 mg of terbinafine hydrochloride ($C_{21}H_{25}N.HCl$), dissolve in methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of terbinafine hydrochloride for assay, previously dried at 105 °C for 4 hours, dissolve in methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the terbinafine peak areas, A_T and A_S , of both solutions.

Amount (mg) of terbinafine hydrochloride (C₂₁H₂₅N.HCl) = $M_{\rm S} \times A_{\rm T}/A_{\rm S} \times 1/4$

 $M_{\rm S}$: Amount (mg) of terbinafine hydrochloride for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 282 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 125 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

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

Mobile phase: A mixture of a solution of tetramethylammonium hydroxide (9 in 2000) adjusted to pH 8.0 with diluted phosphoric acid (1 in 25), acetonitrile and tetrahydrofuran (2:2:1).

Flow rate: Adjust the flow rate so that the retention time of terbinafine is about 8.5 minutes.

System suitability-

System performance: Dissolve 40 mg of terbinafine hydrochloride for assay and 3.5 mg of terphenyl in 200 mL of methanol. When the procedure is run with $10 \,\mu$ L of this solution under the above operating conditions, terphenyl and terbinafine 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 $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of terbinafine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Terbutaline Sulfate



(C₁₂H₁₉NO₃)₂.H₂SO₄: 548.65 5-[(1*RS*)-2-(1,1-Dimethylethylamino)-1-hydroxyethyl]benzene-1,3-diol hemisulfate [*23031-32-5*]

Terbutaline Sulfate contains not less than 98.5% of $(C_{12}H_{19}NO_3)_2$. H_2SO_4 , calculated on the anhydrous basis.

Description Terbutaline Sulfate is white to slightly brownish white crystals or crystalline powder. It is odorless or has a faint odor of acetic acid.

It is freely soluble in water, and practically insoluble in acetonitrile, in ethanol (95), in acetic acid (100), in chloroform, and in diethyl ether.

It 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 reddish purple color is produced.

(2) Determine the absorption spectrum of a solution of

Terbutaline Sulfate in 0.01 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths. This maximum can be biphasic.

(3) A solution of Terbutaline Sulfate (1 in 50) responds to the Qualitative Tests $\langle 1.09 \rangle$ for sulfate.

pH $\langle 2.54 \rangle$ Dissolve 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 slightly yellow.

(2) Chloride $\langle 1.03 \rangle$ —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) Acetic acid—Dissolve 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 sample 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 exactly 2μ L each of the sample solution and standard solution as directed under Gas Chromatography $\langle 2.02 \rangle$ according to the following operating conditions. Measure the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of acetic acid for the two solutions: $A_{\rm T}$ is not larger than $A_{\rm S}$.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 1 m in length, packed with 10% of macrogol 6000 on 180- to 250- μ m terephthalic acid for gas chromatography.

Column temperature: A constant temperature at 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: Mix 0.05 g each of acetic acid (100) and propionic acid in 100 mL of diluted phosphoric acid (59 in 1000). When the procedure is run with $2 \mu L$ of this solution under the above 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 $2 \mu L$ of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of acetic acid is not more than 3.0%.

(4) 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 as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: the absorbance at a wavelength of 330 nm does not exceed 0.47.

(5) Heavy metals <1.07>—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).

(6) Arsenic $\langle 1.11 \rangle$ —Prepare the test solution with 1.0 g

of Terbutaline Sulfate according to method 3, and perform the test (not more than 2 ppm).

Water $\langle 2.48 \rangle$ Not more than 0.5% (1 g, volumetric titration, direct titration).

Residue on ignition $\langle 2.44 \rangle$ 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. Allow to cool, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration, substituting a saturated solution of potassium chloride in methanol for the internal fluid).

Each mL of 0.1 mol/L perchloric acid VS = $54.87 \text{ mg of } (C_{12}H_{19}NO_3)_2.H_2SO_4$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Testosterone Enanthate



 $C_{26}H_{40}O_3$: 400.59 3-Oxoandrost-4-en-17 β -yl heptanoate [315-37-7]

Testosterone Enanthate, when dried, contains not less than 95.0% and not more than 105.0% of $C_{26}H_{40}O_3$.

Description Testosterone Enanthate occurs as white to pale yellow crystals, crystalline powder or a pale yellow-brown, viscous liquid. It is odorless or has a slight, characteristic odor.

It is very soluble in ethanol (95), in 1,4-dioxane and in diethyl 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 on a water bath for 1 hour, cool, and add 10 mL of water. Collect the produced precipitate by suction, wash with water until the last washing is neutral, and dry the precipitate in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours: the precipitate melts $\langle 2.60 \rangle$ between 151°C and 157°C.

Optical rotation $\langle 2.49 \rangle$ [α]_D²⁰: +77 - +88° (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

Purity Acidity—Dissolve 0.5 g of Testosterone Enanthate in 10 mL of ethanol (95) 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 light blue.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (0.5 g, in vacu-

um, phosphorus (V) oxide, 4 hours).

Residue on ignition $\langle 2.44 \rangle$ 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. Measure exactly 10 mL of this solution, and dilute with ethanol (95) to make exactly 100 mL. Measure exactly 10 mL of this solution, and dilute with ethanol (95) to make exactly 100 mL. Perform the test as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$ with this solution. Determine the absorbance A of this solution at the wavelength of maximum absorption at about 241 nm.

> Amount (mg) of $C_{26}H_{40}O_3$ = $A/426 \times 100,000$

Containers and storage Containers—Tight containers. Storage—Light-resistant, and not exceeding 30°C.

Testosterone Enanthate Injection

テストステロンエナント酸エステル注射液

Testosterone Enanthate Injection is an oily solution for injection.

It 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.

Description Testosterone Enanthate Injection is a clear, colorless or pale yellow oily liquid.

Identification Measure a volume of Testosterone Enanthate Injection, equivalent to 0.05 g of Testosterone Enanthate according to the labeled amount, add 8 mL of petroleum ether, and extract with three 10-mL portions of diluted acetic acid (100) (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 on a water bath for 5 minutes. Cool, and add 0.5 mL of iron (III) chloride-acetic acid TS: the color of the solution is blue.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter $\langle 6.06 \rangle$ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter $\langle 6.07 \rangle$ Perform the test according to Method 2: it meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure accurately a volume of Testosterone Enanthate Injection, equivalent to about 25 mg of testosterone enanthate ($C_{26}H_{40}O_3$), and dissolve in chloroform to make exactly 25 mL. Pipet 3 mL of this solution, add chloroform to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Testosterone Propionate RS, proceed in the same manner as for the sample solution, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and standard solution, add exactly 10 mL of isoniazid TS, add methanol to make exactly 20 mL, and allow to stand for 45 minutes. Determine the absorbances, A_T and A_S , of these solutions at 380 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, using a solution obtained by proceeding with 5 mL of chloroform as the blank.

Amount (mg) of testosterone enanthate (
$$C_{26}H_{40}O_3$$
)
= $M_S \times A_T/A_S \times 1.163$

 $M_{\rm S}$: Amount (mg) of Testosterone Propionate RS

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.

Testosterone Propionate

テストステロンプロピオン酸エステル



C₂₂H₃₂O₃: 344.49 3-Oxoandrost-4-en-17β-yl propanoate [57-85-2]

Testosterone Propionate, when dried, contains not less than 97.0% and not more than 103.0% of $C_{22}H_{32}O_3$.

Description Testosterone Propionate occurs as white to pale yellow crystals or crystalline powder.

It is freely soluble in methanol and in ethanol (95), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Testosterone Propionate in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Testosterone Propionate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Testosterone Propionate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Testosterone Propionate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_{D}^{20}$: +83 - +90° (after drying, 0.1 g, ethanol (95), 10 mL, 100 mm).

Melting point <2.60> 118 – 123°C

Purity Related substances—Dissolve 40 mg of Testosterone Propionate in 2 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample 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 Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 10 μ L each of the sample solution and 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 sample solution are not more intense than the spot from the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition $\langle 2.44 \rangle$ 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. To exactly 5 mL of these solutions add exactly 5 mL of the internal standard solution and methanol to make 20 mL, and use these solutions as the sample solution and standard solution. Perform the test with 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of testosterone propionate to that of the internal standard.

Amount (mg) of $C_{22}H_{32}O_3 = M_S \times Q_T/Q_S$

 $M_{\rm S}$: Amount (mg) of Testosterone Propionate RS

Internal standard solution—A solution of progesterone in methanol (9 in 100,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 241 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m 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 testosterone propionate is about 10 minutes.

System suitability—

System performance: When the procedure is run with $5 \,\mu L$ of the standard solution under the above operating conditions, the internal standard and testosterone propionate 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 $5 \,\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the 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.

Testosterone Propionate Injection

テストステロンプロピオン酸エステル注射液

Testosterone Propionate Injection is an oily solution for injection.

It contains not less than 92.5% and not more than 107.5% of the labeled amount of testosterone propionate ($C_{22}H_{32}O_3$: 344.49).

Method of preparation Prepare as directed under Injections, with Testosterone Propionate.

Description Testosterone Propionate Injection is a clear, colorless or pale yellow oily liquid.

Identification Dissolve the residue obtained as directed in the procedure in the Assay in exactly 20 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1 mg of Testosterone Propionate RS in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thinlayer Chromatography $\langle 2.03 \rangle$. Spot 10 μ L each of the sample solution and 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 *R*f values of the principal spot with the sample solution and of the spot with the standard solution are not different each other.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter $\langle 6.06 \rangle$ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> Perform the test according to Method 2: it meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay (i) Chromatographic tube A glass tube about 1 cm in inside diameter and about 18 cm in length, with a glass filter (G3) at the lower end.

(ii) Chromatographic column To about 2 g of silica gel for liquid chromatography add 5 mL of dichloromethane, and mix gently. Transfer and wash into the chromatographic tube with the aid of dichloromethane, allow to elute the dichloromethane through the column, and put a filter paper on the upper end of the silica gel.

(iii) Standard solution Weigh accurately about 10 mg of Testosterone Propionate RS, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and methanol to make 20 mL.

(iv) Sample stock solution To exactly a volume of Testosterone Propionate Injection, equivalent to about 20 mg of testosterone propionate ($C_{22}H_{32}O_3$), add dichloromethane to make 20 mL.

(v) Procedure Transfer exactly 2 mL of the sample stock solution into the chromatographic column, and elute to the upper surface of the silica gel. Wash the inner surface of the chromatographic tube with 15 mL of dichloromethane, elute to the upper surface of the silica gel, and discard the effluent. Elute 15 mL of a mixture of dichloromethane and methanol (39:1), discard the first 5 mL of the effluent, and collect the subsequent effluent. Wash the lower part of the column with a few amount of dichloromethane, combine the washings and the effluent, and evaporate the solvent under reduced pressure. Dissolve the residue so obtained with methanol to make 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and methanol to make 20 mL, and use this solution as the sample solution. Perform the test with 5 μ L each of the sample solution and standard solution as directed in the Assay under Testosterone Propionate.

Amount (mg) of testosterone propionate (C₂₂H₃₂O₃) = $M_{\rm S} \times Q_{\rm T}/Q_{\rm S} \times 2$

 $M_{\rm S}$: Amount (mg) of Testosterone Propionate RS

Internal standard solution—A solution of progesterone in methanol (9 in 100,000).

Containers and storage Containers-Hermetic containers.

Freeze-dried Tetanus Antitoxin, Equine

乾燥破傷風ウマ抗毒素

Freeze-dried Tetanus Antitoxin, Equine, is a preparation for injection which is dissolved before use.

It contains tetanus antitoxin in immunoglobulin of horse origin.

It conforms to the requirements of Freeze-dried Tetanus Antitoxin, Equine, in the Minimum Requirements for Biological Products.

Description Freeze-dried Tetanus Antitoxin, Equine, becomes a clear, colorless to light yellow-brown liquid or slightly white-turbid liquid on addition of solvent.

Adsorbed Tetanus Toxoid

沈降破傷風トキソイド

Adsorbed Tetanus Toxoid is a liquid for injection containing tetanus toxoid prepared by treating tetanus toxin with formaldehyde by a method involving no appreciable loss of the immunogenicity and rendered insoluble by the addition of aluminum salt.

It conforms to the requirements of Adsorbed Tetanus Toxoid in the Minimum Requirements for Biological Products.

Description Adsorbed Tetanus Toxoid becomes a uniform white-turbid liquid on shaking.

Tetracaine Hydrochloride



C₁₅H₂₄N₂O₂.HCl: 300.82 2-(Dimethylamino)ethyl 4-(butylamino)benzoate monohydrochloride [*136-47-0*]

Tetracaine Hydrochloried, when dried, contains not less than 98.5% of $C_{15}H_{25}N_2O_2$.HCl.

Description Tetracaine Hydrochloride occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste followed by a sense of numbress on the tongue.

It 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 diethyl 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, and allow to stand in a cold place. Collect the precipitate, wash with water until the washings is neutral, and dry in a desiccator (silica gel) for 24 hours: it melts $\langle 2.60 \rangle$ 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. Collect the precipitate, recrystallize from water, and dry at 80°C for 2 hours: it melts $\langle 2.60 \rangle$ between 130°C and 132°C.

(3) Determine the absorption spectrum of a solution of Tetracaine Hydrochloride in ethanol (99.5) (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) A solution of Tetracaine Hydrochloride (1 in 10) responds to the Qualitative Tests $\langle 1.09 \rangle$ for chloride.

Purity Heavy metals $\langle 1.07 \rangle$ —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).

Loss on drying $\langle 2.41 \rangle$ Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 on a water bath for 15 minutes, cool, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction. Each mL of 0.1 mol/L perchloric acid VS = $30.08 \text{ mg of } C_{15}H_{24}N_2O_2.HCl$

Containers and storage Containers—Tight containers.

Tetracycline Hydrochloride

テトラサイクリン塩酸塩



 $\begin{array}{l} C_{22}H_{24}N_2O_8.HCl:\ 480.90\\ (4S,4aS,5aS,6S,12aS)-4-Dimethylamino-\\ 3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-\\ 1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-\\ carboxamide monohydrochloride\\ [64-75-5]\end{array}$

Tetracycline Hydrochloride is the hydrochloride of a tetracycline substance having antibacterial activity produced by the growth of *Streptomyces aureofaciens*.

It contains not less than $950 \,\mu g$ (potency) and not more than $1010 \,\mu g$ (potency) per mg, calculated on the dried basis. The potency of Tetracycline Hydrochloride is expressed as mass (potency) of tetracycline hydrochloride (C₂₂H₂₄N₂O₈.HCl).

Description Tetracycline Hydrochloride occurs as a yellow to pale brownish yellow crystalline powder.

It is freely soluble in water, and sparingly soluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Tetracycline Hydrochloride (1 in 62,500) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Tetracycline Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tetracycline Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Tetracycline Hydrochloride RS: 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 $\langle 1.09 \rangle$ (2) for chloride.

pH $\langle 2.54 \rangle$ Dissolve 1.0 g of Tetracycline Hydrochloride in 100 mL of water: the pH of the solution is between 1.8 and 2.8.

Purity (1) Heavy metals $\langle 1.07 \rangle$ —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 $\langle 1.11 \rangle$ —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—Dissolve 25 mg of Tetracycline Hydrochloride in 50 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Pipet 3 mL of the sample 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 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the areas of each peak by the automatic integration method: each peak area other than tetracycline from the sample solution is not larger than the peak area of tetracycline from the standard solution, and the total area of the peaks other than tetracycline from the sample solution is not larger than 3 times of the peak area of tetracycline 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.

Time span of measurement: About 7 times as long as the retention time of tetracycline beginning after the solvent peak.

System suitability—

Test for required detection: Pipet 3 mL of the standard solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and confirm that the peak area of tetracycline obtained from $20 \,\mu$ L of this solution is equivalent to 1 to 5% of that from $20 \,\mu$ L of the standard solution.

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

System repeatability: When the test is repeated 6 times with $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of tetracycline is not more than 1.0%.

Loss on drying $\langle 2.41 \rangle$ Not more than 2.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.3% (1.0 g).

Assay Weigh accurately an amount of Tetracycline Hydrochloride and Tetracycline Hydrochloride RS, equivalent to about 25 mg (potency), and dissolve each in 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μ L each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak area, A_T and A_S , of tetracycline of each solution.

> Amount [μ g (potency)] of C₂₂H₂₄N₂O₈.HCl = $M_{\rm S} \times A_{\rm T}/A_{\rm S} \times 1000$

 $M_{\rm S}$: Amount [mg (potency)] of Tetracycline Hydrochloride RS

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with stylene-divinylbenzene copolymer for liquid chromatography (0.01 μ m in pore diameter).

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

JP XVI

Mobile phase: Dissolve 3.5 g of dipotassium hydrogenphosphate, 2.0 g of tetrabutylammonium hydrogensulfate and 0.4 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 300 mL of water, adjust to pH 9.0 with sodium hydroxide TS, add 90.0 g of *t*-butanol, 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—

System performance: Dissolve 0.05 g of Tetracycline Hydrochloride RS in water to make 25 mL. Heat 5 mL of this solution on a water bath for 60 minutes, then add water to make 25 mL. When the procedure is run with $20 \,\mu$ L 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 $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of tetracycline is not more than 1.0%.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Thallium (²⁰¹Tl) Chloride Injection

塩化タリウム (²⁰¹TI) 注射液

Thallium (²⁰¹Tl) Chloride Injection is an aqueous solution for injection

It contains thallium-201 (²⁰¹Tl) in the form of thallous chloride.

It conforms to the requirements of Thallium (²⁰¹Tl) Chloride Injection in the Minimum Requirements for Radiopharmaceuticals.

Test for Extractable Volume of Parenteral Preparations and Insoluble Particulate Matter Test for Injections are not applied to this injection.

Description Thallium (²⁰¹Tl) Chloride Injection is a clear, colorless liquid.

Theophylline

テオフィリン



C₇H₈N₄O₂: 180.16 1,3-Dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione [58-55-9]

The ophylline, when dried, contains not less than 99.0% of $C_7H_8N_4O_2$.

Description Theophylline occurs as white crystals or crystalline powder.

It is soluble in *N*,*N*-dimethylformamide, and slightly soluble in water and in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS.

Identification (1) Determine the absorption spectrum of a solution of Theophylline in 0.1 mol/L hydrochloric acid TS (1 in 200,000) as directed under Ultraviolet-visible Spectro-photometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Theophylline, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 271 – 275°C

Purity (1) Acidity—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 develops.

(2) Heavy metals $\langle 1.07 \rangle$ —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 $\langle 1.11 \rangle$ —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 sample solution. Pipet 1 mL of the sample 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 $\langle 2.03 \rangle$. Spot 10 μ L each of the sample 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, 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 sample solution are not more intense than the spot from the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.25 g of Theophylline, previously dried, and dissolve in 100 mL of water, add exactly 20 mL of 0.1 mol/L silver nitrate VS, shake the mixture, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

> Each mL of 0.1 mol/L sodium hydroxide VS = 18.02 mg of $C_7H_8N_4O_2$

Containers and storage Containers-Well-closed containers.

Thiamazole

チアマゾール

C₄H₆N₂S: 114.17 1-Methyl-1*H*-imidazole-2-thiol [60-56-0]

Thiamazole, when dried, contains not less than 98.0% of $C_4H_6N_2S$.

Description Thiamazole occurs as white to pale yellowish white crystals or crystalline powder. It has a faint, characteristic odor, and has a bitter taste.

It is freely soluble in water and in ethanol (95), and slightly soluble in diethyl ether.

The pH of the solution (1 in 50) 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 develops, 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 develops.

Melting point <2.60> 144 – 147°C

Purity (1) Selenium—Proceed with 0.10 g of Thiamazole as directed under Oxygen Flask Combustion Method <1.06>, using 25 mL of diluted nitric acid (1 in 30) 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 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 sample 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 5 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 sample solution and 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 hydroxylammonium chloride, shake gently to dissolve. To these solutions add 5 mL of a solution prepared by dissolving 0.10 g of 2,3diaminonaphthalene and 0.5 g of hydroxylammonium chloride in 0.1 mol/L hydrochloric acid TS to make 100 mL, shake, and allow to stand for 100 minutes. Transfer these solutions to corresponding separators, rinse the beakers with 10 mL of water, combine the rinsings in the respective separators, 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

these solutions as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, using a solution prepared with 40 mL of diluted nitric acid (1 in 60) in the same manner as the blank. The absorbance of of the sample solution at the wavelength of maximum absorbance at about 378 nm does not exceed the absorbance of the standard solution.

(2) Heavy metals $\langle 1.07 \rangle$ —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).

(3) Arsenic $\langle 1.11 \rangle$ —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 $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 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 $\langle 2.50 \rangle$ with 0.1 mol/L sodium hydroxide VS, until a persistent blue-green color is produced. Determine the total volume of 0.1 mol/L sodium hydroxide VS consumed.

Each mL of 0.1 mol/L sodium hydroxide VS = 11.42 mg of C₄H₆N₂S

Containers and storage Containers-Well-closed containers.

Storage—Light-resistant.

Thiamazole Tablets

チアマゾール錠

Thiamazole Tablets contain not less than 94.0% and not more than 106.0% of the labeled amount of thiamazole (C₄H₆N₂S: 114.17).

Method of preparation Prepare as directed under Tablets, with Thiamazole.

Identification (1) To a quantity of powdered Thiamazole Tablets, equivalent to 0.05 g of Thiamazole according to the labeled amount, add 20 mL of hot ethanol (95), 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 sample solution. To 1 mL of the sample solution add 1 mL of sodium hydroxide TS, shake, and add 3 drops of sodium pentacyanonitrosylferrate (III) TS: a yellow color develops, 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 sample solution obtained in (1), proceed as directed in the Identification (2) under Thiamazole.

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, pipet 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 $\langle 2.50 \rangle$ with 0.1 mol/L sodium hydroxide VS. Continue the titration until a persistent bluegreen 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 = $11.42 \text{ mg of } C_4H_6N_2S$

Containers and storage Containers-Well-closed containers.

Storage-Light-resistant.

Thiamine Chloride Hydrochloride

Vitamin B₁ Hydrochloride

チアミン塩化物塩酸塩



C₁₂H₁₇ClN₄OS.HCl: 337.27 3-(4-Amino-2-methylpyrimidin-5-ylmethyl)-5-(2hydroxyethyl)-4-methylthiazolium chloride monohydrochloride [67-03-8]

Thiamine Chloride Hydrochloride contains not less than 98.5% of $C_{12}H_{17}ClN_4OS.HCl$, calculated on the anhydrous basis.

Description Thiamine Chloride Hydrochloride occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor.

It is freely soluble in water, sparingly soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 245°C (with decomposition).

Identification (1) To 5 mL of a solution of Thiamine Chloride 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 it is again made alkaline.

(2) Determine the absorption spectrum of a solution of Thiamine Chloride Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Thiamine Chloride Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Thiamine Chloride Hydrochloride, previously dried at 105° C for 2 hours, as directed in the potassium bromide disk method under the Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum, or the spectrum of Thiamine Chloride Hydrochloride RS previously driedat 105° C for 2 hours: both spectra exhibit similar intensities of absorption at the same wave numbers. In case when some differences are found between the spectra, repeat the test with residues obtained by dissolving these substances in water, evaporating to dryness, and drying at 105° C for 2 hours.

(4) A solution of Thiamine Chloride Hydrochloride (1 in 500) responds to the Qualitative Tests $\langle 1.09 \rangle$ for chloride.

pH $\langle 2.54 \rangle$ Dissolve 1.0 g of Thiamine Chloride 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 Chloride 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 dichromate VS add water to make 1000 mL.

(2) Sulfate $\langle 1.14 \rangle$ —Weigh 1.5 g of Thiamine Chloride 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 Chloride 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 produced at the junction of the two layers.

(4) Heavy metals $\langle 1.07 \rangle$ —Proceed with 1.0 g of Thiamine Chloride 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 Chloride Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution, as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and determine the area of each peak by the automatic integration method: the total area of the peaks other than thiamine obtained from sample solution is not larger than the peak area of thiamine 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.

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

System suitability-

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 $10 \,\mu$ L of this solution is equivalent to 7 to 13% of that from $10 \,\mu$ L of the standard solution.

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

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ 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%.

Water $\langle 2.48 \rangle$ Not more than 5.0% (30 mg, coulometric titration).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.2% (1 g).

Assay Weigh accurately about 0.1 g each of Thiamine Chloride Hydrochloride and Thiamine Chloride Hydrochloride RS (separately determine the water $\langle 2.48 \rangle$ in the same manner as Thiamine Chloride Hydrochloride), and dissolve them in the mobile phase to make exactly 50 mL. To 10 mL each of the solutions, accurately measured, add exactly 5 mL each of the internal standard solution, add the mobile phase to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with $10 \,\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of thiamine to that of the internal standard.

Amount (mg) of
$$C_{12}H_{17}CIN_4OS.HCl= M_S \times Q_T/Q_S$$

 $M_{\rm S}$: Amount (mg) of Thiamine Chloride Hydrochloride RS, calculated on the anhydrous basis

Internal standard solution—A solution methyl benzoate in methanol (1 in 50).

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-length: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m 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 μ L 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 $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio 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.

Thiamine Chloride Hydrochloride Injection

Vitamin B₁ Hydrochloride Injection

チアミン塩化物塩酸塩注射液

Thiamine Chloride Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 115.0% of the labeled amount of thiamine Chloride hydrochloride ($C_{12}H_{17}ClN_4OS.HCl:$ 337.27).

Method of preparation Prepare as directed under Injections, with Thiamine Chloride Hydrochloride.

Description Thiamine Chloride Hydrochloride Injection is a clear, colorless liquid.

pH: 2.5 – 4.5

Identification To a volume of Thiamine Chloride Hydrochloride Injection, equivalent to 0.05 g of Thiamine Chloride 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 Chloride Hydrochloride.

Bacterial endotoxins <4.01> Less than 6.0 EU/mg.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Dilute with 0.001 mol/L hydrochloric acid TS if necessary, then measure exactly a volume of Thiamine Chloride Hydrochloride Injection, equivalent to about 20 mg of thiamine chloride hydrochloride (C12H17ClN4OS.HCl), and add 20 mL of methanol and 0.001 mol/L hydrochloric acid TS to make 100 mL. To 25 mL of this solution, exactly measured, add exactly 5 mL of the internal standard solution, add 0.001 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Thiamine Chloride Hydrochloride RS (separately determine the water $\langle 2.48 \rangle$ in the same manner as Thiamine Chloride Hydrochloride), and dissolve in 0.001 mol/L hydrochloric acid TS to make exactly 50 mL. To 10 mL of this solution, exactly measured, add 20 mL of methanol and 0.001 mol/L hydrochloric acid TS to make exactly 100 mL. To 25 mL of this solution, exactly measured, add exactly 5 mL of the internal standard solution, add 0.001 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Thiamine Chloride Hydrochloride.

Amount (mg) of thiamine chloride hydrochloride (C₁₂H₁₇ClN₄OS.HCl) = $M_{\rm S} \times Q_{\rm T}/Q_{\rm S} \times 1/5$

M_S: Amount (mg) of Thiamine Chloride Hydrochloride

JP XVI

RS, calculated on the anhydrous basis

Internal standard solution—A solution of methyl benzoate in methanol (1 in 200).

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.

Thiamine Chloride Hydrochloride Powder

Vitamin B₁ Hydrochloride Powder

チアミン塩化物塩酸塩散

Thiamine Chloride Hydrochloride Powder contains not less than 95.0% and not more than 115.0% of the labeled amount of thiamine chloride hydrochloride ($C_{12}H_{17}ClN_4OS.HCl:$ 337.27).

Method of preparation Prepare as directed under Powders, with Thiamine Chloride Hydrochloride.

Identification To a portion of Thiamine Chloride Hydrochloride Powder, equivalent to 0.02 g of Thiamine Chloride Hydrochloride according to the labeled amount, add 50 mL of water and 10 mL of dilute acetic acid, shake, and filter. Proceed with 5 mL of the filtrate as directed in the Identification (1) under Thiamine Chloride Hydrochloride.

Purity Rancidity—Thiamine Chloride Hydrochloride Powder has no unpleasant or rancid odor. It is tasteless.

Assay Weigh accurately a quantity of Thiamine Chloride Hydrochloride Powder, equivalent to about 20 mg of thiamine chloride hydrochloride (C12H17ClN4OS.HCl), add 60 mL of 0.01 mol/L hydrochloric acid TS, and heat on 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 supernatant liquid, add exactly 5 mL of the internal standard solution, add water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Thiamine Chloride Hydrochloride RS (separately determine the water $\langle 2.48 \rangle$ in the same manner as Thiamine Chloride Hydrochloride), and dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. To 10 mL of this solution, exactly measured, add 50 mL of 0.01 mol/L hydrochloric acid TS, and add methanol to make exactly 100 mL. To 25 mL of this solution, exactly measured, add exactly 5 mL of the internal standard solution, add water to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Thiamine Chloride Hydrochloride.

Amount (mg) of thiamine chloride hydrochloride ($C_{12}H_{17}CIN_4OS.HCl$) = $M_S \times Q_T/Q_S \times 1/5$

 $M_{\rm S}$: Amount (mg) of Thiamine Chloride Hydrochloride RS, calculated on the anhydrous basis

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 B₁ Nitrate



 $C_{12}H_{17}N_5O_4S:$ 327.36 3-(4-Amino-2-methylpyrimidin-5-ylmethyl)-5-(2hydroxyethyl)-4-methylthiazolium nitrate [532-43-4]

Thiamine Nitrate, when dried, contains not less than 98.0% and not more than 102.0% of $C_{12}H_{17}N_5O_4S$.

Description Thiamine Nitrate occurs as white crystals or crystalline powder. It is odorless or a slight, characteristic odor.

It is sparingly soluble in water, and very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 193°C (with decomposition).

Identification (1) Take 2-mL portions 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 (II) 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 again made alkaline.

(4) A solution of Thiamine Nitrate (1 in 50) responds to the Qualitative Tests $\langle 1.09 \rangle$ (1) and (2) for nitrate.

pH $\langle 2.54 \rangle$ 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 $\langle 1.03 \rangle$ —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 $\langle 1.14 \rangle$ —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%).

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(3) Heavy metals $\langle 1.07 \rangle$ —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).

Loss on drying $\langle 2.41 \rangle$ Not more than 1.0% (0.5 g, 105°C, 2 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.2% (1 g).

Assay Weigh accurately about 0.1 g each of Thiamine Nitrate, previously dried, and Thiamine Chloride Hydrochloride RS (separately determine the water $\langle 2.48 \rangle$ in the same manner as Thiamine Chloride Hydrochloride), and dissolve them in the mobile phase to make exactly 50 mL. To 10 mL each of the solutions, accurately measured, add exactly 5 mL each of the internal standard solution, add the mobile phase to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with $10 \,\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of thiamine to that of the internal standard.

Amount (mg) of
$$C_{12}H_{17}N_5O_4S = M_8 \times Q_T/Q_8 \times 0.971$$

 $M_{\rm S}$: Amount (mg) of Thiamine Chloride Hydrochloride RS, calculated on the anhydrous basis

Internal standard solution—A solution of methyl benzoate in methanol (1 in 50).

Operating conditions—

Detector: An ultraviolet spectrophotometer (wavelength: 254 nm).

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

Column temperature: A constant temperature of about $30^{\circ}C$.

Mobile phase: Dissolve 1.1 g of sodium l-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 μ L 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 $10 \,\mu\text{L}$ 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.

Thiamylal Sodium



C₁₂H₁₇N₂NaO₂S: 276.33 Monosodium 5-allyl-5-[(1*RS*)-1-methylbutyl]-4,6dioxo-1,4,5,6-tetrahydropyrimidine-2-thiolate [*337-47-3*]

Thiamylal Sodium contains not less than 97.5% and not more than 101.0% of $C_{12}H_{17}N_2NaO_2S$, calculated on the dried basis.

Description Thiamylal Sodium occurs as light yellow crystals or powder.

It is very soluble in water, and freely soluble in ethanol (95).

The pH of a solution of Thiamylal Sodium (1 in 10) is between 10.0 and 11.0.

It is hygroscopic.

It is gradually decomposed by light.

Its solution in ethanol (95) (1 in 10) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Thiamylal Sodium in ethanol (95) (7 in 1,000,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Thiamylal Sodium, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Thiamylal Sodium (1 in 10) responds to Qualitative Tests $\langle 1.09 \rangle$ 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 light yellow.

(2) Heavy metals $\langle 1.07 \rangle$ —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 0.10 g of Thiamylal Sodium in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL and 3 mL of the sample 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 $\langle 2.03 \rangle$. Spot 10 μ L each of the sample solution and 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 for a night: the spot appeared around Rf value 0.1 obtained with the sample 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 sample solution is not more intense than the spot with the standard solution (1).

Loss on drying $\langle 2.41 \rangle$ 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 200 mL, and use this solution as the sample 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 200 mL, and use this solution as the standard solution. Perform the test with $20 \,\mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of thiamylal to that of the internal standard.

> Amount (mg) of $C_{12}H_{17}N_2NaO_2S$ = $M_S \times Q_T/Q_S \times 10 \times 1.086$

 $M_{\rm S}$: Amount (mg) of Thiamylal RS

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 inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m 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 μ L 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 $20 \,\mu\text{L}$ 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

注射用チアミラールナトリウム

Thiamylal Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of thiamylal sodium ($C_{12}H_{17}N_2NaO_2S$: 276.33).

Method of preparation Prepare as directed under Injections, with 100 parts of Thiamylal Sodium and 7 parts of Dried Sodium Carbonate in mass.

Description Thiamylal Sodium for Injection occurs as light yellow crystals, powder or masses.

It is hygroscopic.

It 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 precipitate so obtained in 1 mL of water, and add 1 mL of barium chloride TS: a white precipitate is produced. Centrifuge this solution, take off the 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 $\langle 2.24 \rangle$: it exhibits maxima between 236 nm and 240 nm, and between 287 nm and 291 nm.

pH $\langle 2.54 \rangle$ The pH of a solution obtained by dissolving 1.0 g of Thiamylal Sodium for Injection in 40 mL of water 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 sample solution. Proceed as diected in the Purity (3) under Thiamylal Sodium.

Bacterial endotoxins <4.01> Less than 1.0 EU/mg.

Uniformity of dosage units $\langle 6.02 \rangle$ It meets the requirement of the Mass variation test.

Foreign insoluble matter $\langle 6.06 \rangle$ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: 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 (C₁₂H₁₇N₂NaO₂S). 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 200 mL, and use this solution as the sample solution. Proceed the test with the sample solution as directed in the Assay under Thiamylal Sodium.

Amount (mg) of thiamylal sodium ($C_{12}H_{17}N_2NaO_2S$)

in 1 container = $M_{\rm S} \times Q_{\rm T}/Q_{\rm S} \times V/50 \times 1.086$

 $M_{\rm S}$: Amount (mg) of Thiamylal RS

Internal standard solution—A solution of phenyl benzoate in methanol (3 in 500).

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.

Thianthol

チアントール

Thianthol consists of dimethylthianthrene and ditoluene disulfide.

It contains not less than 23.5% and not more than 26.5% of sulfur (S: 32.07).

Description Thianthol is a yellowish, viscous liquid. It has a faint, agreeable odor.

It is freely soluble in diethyl ether, slightly soluble in ethanol (95), and practically insoluble in water.

It, when cold, may separate crystals, which melt on warming.

Specific gravity d_{20}^{20} : 1.19 – 1.23

Identification To 0.1 g of Thianthol add cautiously 5 mL of sulfuric acid: a blue-purple color develops. Add 5 to 6 drops of nitric acid to the solution: the color of the solution changes to yellow-red with evolution of gas.

Purity (1) Acidity or alkalinity—Shake 10 g of Thianthol with 20 mL of water, allow to stand, and separate the water layer. The solution is neutral.

(2) Sulfate—To 10 mL of the water layer obtained in (1) add 2 to 3 drops of barium chloride TS: no opalescence is produced.

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 10 mg of Thianthol, and proceed as directed in the sulfur determination of Oxygen Flask Combustion Method $\langle 1.06 \rangle$, using a mixture of 5 mL of diluted sodium hydroxide TS (1 in 10) and 1.0 mL of hydrogen peroxide TS as an absorbing liquid.

Containers and storage Containers—Tight containers.

Compound Thianthol and Salicylic Acid Solution

複方チアントール・サリチル酸液

Compound Thianthol and Salicylic Acid Solution contains not less than 1.8 w/v% and not more than 2.2 w/v% of salicylic acid (C₇H₆O₃: 138.12), and not less than 1.8 w/v% and not more than 2.2 w/v% of phenol (C₆H₆O: 94.11).

Method of preparation

	2 00 T
Thianthol	200 mL
Salicylic Acid	20 g
Phenol	20 g
Olive Oil	50 mL
Ether	100 mL
Petroleum Benzin	a sufficient quantity
	T 1 1000 I

To make 1000 mL

Dissolve Salicylic Acid and Phenol in Ether, add Thianthol, Olive Oil and Petroleum Benzin to this solution, mix and dissolve to make 1000 mL.

Description Compound Thianthol and Salicylic Acid Solution is a light yellow liquid, having a characteristic odor.

Identification (1) Place 1 mL of Compound Thianthol and Salicylic Acid Solution to a porcelain dish, and evaporate on a water bath to dryness. To the residue add cautiously 5 mL of sulfuric acid: the color of the solution changes to yellow-red with evolution of gas (thianthol).

(2) Shake 10 mL of Compound Thianthol and Salicylic Acid Solution with 10 mL of sodium hydrogen carbonate TS, and separate the water layer. To 0.5 mL of the water layer add hydrochloric acid-potassium chloride buffer solution, pH 2.0, to make 50 mL, and to 5 mL of this solution add 5 mL of a solution of iron (III) nitrate enneahydrate (1 in 200): a red-purple color is produced (salicylic acid).

(3) Wash the upper phase obtained in (2) with 10 mL of sodium hydrogen carbonate TS, and extract with 10 mL of dilute sodium hydroxide TS. Shake 1 mL of the extract with 1 mL of sodium nitrate TS and 1 mL of dilute hydrochloric acid, and add 3 mL of sodium hydroxide TS: a yellow color is produced (phenol).

(4) To 1 mL of Compound Thianthol and Salicylic Acid Solution add 10 mL of ethanol (95), mix, and use this solution as the sample solution. Dissolve 0.01 g each of salicylic acid, phenol and thianthol in 5 mL each of ethanol (95), and use each solution as standard solutions (1), (2) and (3). Perform the test with these solutions as directed under Thinlayer Chromatography $\langle 2.03 \rangle$. Spot 5 μ L each of the sample solution and standard solutions on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (45:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): three spots obtained from the sample solution and the corresponding spots of standard solutions (1), (2) and (3) show the same Rf value. Spray evenly iron (III) chloride TS on the plate: the spot from standard solution (1) and the corresponding spot from the sample solution reveal a purple color.

Assay Measure exactly 2 mL of Compound Thianthol and Salicylic Acid Solution, add exactly 10 mL of the internal standard solution, then add 70 mL of diluted methanol (1 in 2), mix well, and add diluted methanol (1 in 2) to make 100 mL. Filter, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Weigh accurately about 0.2 g of salicylic acid for assay, previously dried in a desiccator (silica gel) for 3 hours, and about 0.2 g of phenol for assay, dissolve in diluted methanol (1 in 2) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and diluted methanol (1 in 2) to make 100 mL, and use this solution as the standard solution. With 5 μ L each of the sample solution and standard solution, perform the test as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and calculate the ratios, Q_{Ta} and Q_{Tb} , of the peak area of salicylic acid and phenol to that of the internal standard in the sample solution, and the ratios, Q_{Sa} and Q_{Sb} , of the peak area of salicylic acid and phenol to that of the internal standard ard in the standard solution.

Amount (mg) of salicylic acid
$$(C_7H_6O_3)$$

= $M_{Sa} \times Q_{Ta}/Q_{Sa} \times 1/5$
Amount (mg) of phenol (C_6H_6O)
= $M_{Sb} \times Q_{Tb}/Q_{Sb} \times 1/5$

 M_{Sa} : Amount (mg) of salicylic acid for assay M_{Sb} : Amount (mg) of phenol for assay

Internal standard solution—A solution of theophylline in methanol (1 in 10,000).

Operating conditions—

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

Column: A stainless steel column about 4 mm in inside diameter and 25 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: Room temperature.

Mobile phase: A mixture of 0.1 mol/L phosphate buffer solution, pH 7.0, and methanol (3:1).

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

Selection of column: Dissolve 0.2 g of benzoic acid, 0.2 g of salicylic acid and 0.05 g of theophylline in 100 mL of diluted methanol (1 in 2). To 10 mL of this solution add 90 mL of diluted methanol (1 in 2). Proceed with $10 \,\mu$ L of this solution under the above operating conditions. Use a column giving elution of benzoic acid, salicylic acid and theophylline in this order, and clearly dividing each peak.

Containers and storage Containers—Tight containers. Storage—Light-resistant, and not exceeding 25°C.

Thiopental Sodium

チオペンタールナトリウム



C₁₁H₁₇N₂NaO₂S: 264.32 Monosodium 5-ethyl-5-[(1*RS*)-1-methylbutyl]-4,6dioxo-1,4,5,6-tetrahydropyrimidine-2-thiolate [*71-73-8*]

Thiopental Sodium, when dried, contains not less than 97.0% of $C_{11}H_{17}N_2NaO_2S$.

Description Thiopental Sodium occurs as a light yellow powder. It has a faint, characteristic odor.

It is very soluble in water, freely soluble in ethanol (95), and practically insoluble in diethyl ether.

A solution of Thiopental Sodium (1 in 10) is alkaline. It is hygroscopic.

Its solution 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 (II) 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 $\langle 1.09 \rangle$ 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 portions of chloroform. Combine the chloroform extracts, evaporate on a water bath, and dry at 105 °C for 2 hours: the residue melts $\langle 2.60 \rangle$ between 157 °C and 162 °C.

(3) A solution of Thiopental Sodium (1 in 10) responds to the Qualitative Tests $\langle 1.09 \rangle$ (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 light yellow.

(2) Heavy metals $\langle 1.07 \rangle$ —Dissolve 2.0 g of Thiopental 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 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 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 portions 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 sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and the standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Measure each peak area of each solution by the automatic integration method: the total area of peaks other than thiopental from the sample solution is not larger than the peak area of thiopental from the standard solution.

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel (5 μ m 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 the pH to 3.0 with phosphoric acid. 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.

Time span of measurement: About 1.5 times as long as the retention time of thiopental.

System suitability—

Test for required detection: 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 μ L of this solution is equivalent to 15 to 25% of that from 20 μ L 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 μ L of this solution under the above operating conditions, isopropyl parahydroxybenzoate and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 1.9.

System repeatability: When the test is repeated 6 times with $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of thiopental is not more than 2.0%.

Loss on drying $\langle 2.41 \rangle$ 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 separator, 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 portions of chloroform. Combine the chloroform extracts, wash with two 5-mL portions of water, and extract the washings with two 10-mL portions of chloroform. Filter the combined chloroform extracts into a conical flask, and wash the filter paper with three 5-mL portions of chloroform. Combine the filtrate and the washings, and add 10 mL of ethanol (95). Titrate <2.50> with 0.1 mol/L potassium hydroxide-ethanol VS until the color of the solution changes from yellow through light 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 = $26.43 \text{ mg of } C_{11}H_{17}N_2NaO_2S$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Thiopental Sodium for Injection

注射用チオペンタールナトリウム

Thiopental Sodium for Injection is a preparation for injection which is dissolved before use.

It 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 in mass.

Description Thiopental Sodium for Injection is a light yel-

low powder or mass, and has a slight, characteristic odor.

It is very soluble in water, and practically insoluble in dehydrated diethyl ether.

It 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 formed. 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 $\langle 2.54 \rangle$ Dissolve 1.0 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 $\langle 2.41 \rangle$ Not more than 2.0% (1 g, in vacuum, 80°C, 4 hours).

Bacterial endotoxins <4.01> Less than 0.30 EU/mg.

Uniformity of dosage units $\langle 6.02 \rangle$ It meets the requirement of the Mass variation test.

Foreign insoluble matter $\langle 6.06 \rangle$ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

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 (VmL) of this solution, equivalent to about 15 mg of thiopental sodium (C11H17N2NaO2S), 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 sample solution. Separately, weigh accurately about 46 mg of thiopental for assay, 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 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, $A_{\rm T}$ and $A_{\rm S}$, at 304 nm.

Amount (mg) of thiopental sodium (C₁₁H₁₇N₂NaO₂S) in each sample of Thiopental Sodium for Injection = $M_{\rm S} \times A_{\rm T}/A_{\rm S} \times 300/V \times 1.091$

 $M_{\rm S}$: Amount (mg) of thiopental sodium for assay

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.

Thioridazine Hydrochloride

チオリダジン塩酸塩



 $C_{21}H_{26}N_2S_2.HCl: 407.04$ 10-{2-[(2RS)-1-Methylpiperidin-2-yl]ethyl}-2methylsulfanyl-10H-phenothiazine monohydrochloride [130-61-0]

Thioridazine Hydrochloride, when dried, contains not less than 99.0% of $C_{21}H_{26}N_2S_2$.HCl.

Description Thioridazine Hydrochloride occurs as a white to pale yellow, crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in water, in methanol, in ethanol (95) and in acetic acid (100), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Thioridazine Hydrochloride (1 in 100) is between 4.2 and 5.2.

It is gradually colored by light.

Identification (1) Dissolve 0.01 g of Thioridazine Hydrochloride in 2 mL of sulfuric acid: a deep blue color develops.

(2) Dissolve 0.01 g of Thioridazine Hydrochloride in 2 mL of water, and add 1 drop of cerium (IV) tetraammonium sulfate TS: a blue color develops, and the color disappears on the addition of excess of the reagent.

(3) Determine the infrared absorption spectrum of Thioridazine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectro-photometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) To 5 mL of a solution of Thioridazine Hydrochloride (1 in 100) add 2 mL of ammonia TS, and heat on a water bath for 5 minutes. After cooling, filter, and acidify the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests $\langle 1.09 \rangle$ (2) for chloride.

Melting point <2.60> 159 – 164°C

Purity (1) Heavy metals $\langle 1.07 \rangle$ —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) Arsenic $\langle 1.11 \rangle$ —Prepare the test solution with 1.0 g of Thioridazine Hydrochloride, according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Conduct this procedure under the protection from the sunlight. Dissolve 0.10 g of Thioridazine Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and 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 these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. 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. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 40.70 mg of $C_{21}H_{26}N_2S_2$.HCl

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Thiotepa

チオテパ



C₆H₁₂N₃PS: 189.22 Tris(aziridin-1-yl)phosphine sulfide [52-24-4]

Thiotepa, when dried, contains not less than 98.0% of $C_6H_{12}N_3PS$.

Description Thiotepa occurs as colorless or white crystals, or white, crystalline powder. It is odorless.

It is freely soluble in water, in ethanol (95) and in diethyl ether.

A solution of Thiotepa (1 in 10) is neutral.

Identification (1) To 5 mL of a solution of Thiotepa (1 in 100) add 1 mL of hexaammonium heptamolybdate 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 $\langle 1.09 \rangle$ (2) for phosphate.

(3) Dissolve 0.1 g of Thiotepa in a mixture of 1 mL of lead (II) 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 <2.60> 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 col-

orless.

(2) Heavy metals <1.07>—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 $\langle 1.11 \rangle$ —Dissolve 0.20 g of Thiotepa in 5 mL of water, and add 1 mL of nitric acid and 1 mL of sulfuric acid. Take this solution, prepare the test solution according to Method 2, and perform the test (not more than 10 ppm).

Loss on drying $\langle 2.41 \rangle$ Not more than 0.20% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 $\langle 2.50 \rangle$ the excess sulfuric acid with 0.1 mol/L sodium hydroxide VS until the color of the solution changes from red to light yellow (indicator: 3 drops of methyl red TS). Perform a blank determination.

Each mL of 0.05 mol/L sulfuric acid VS = 6.307 mg of C₆H₁₂N₃PS

Containers and storage Containers—Tight containers. Storage—Light-resistant, and in a cold place.

L-Threonine

L-トレオニン



 $C_4H_9NO_3$: 119.12 (2*S*,3*R*)-2-Amino-3-hydroxybutanoic acid [72-19-5]

L-Threonine, when dried, contains not less than 98.5% of C₄H₉NO₃.

Description L-Threonine occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor, and has a slightly sweet taste.

It is freely soluble in formic acid, soluble in water, and practically insoluble in ethanol (95).

Identification Determine the infrared absorption spectrum of L-Threonine, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_{D}^{20}$: $-26.0 - 29.0^{\circ}$ (after drying, 1.5 g, water, 25 mL, 100 mm).

pH $\langle 2.54 \rangle$ Dissolve 0.20 g of L-Threonine in 20 mL of water: 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 $\langle 1.03 \rangle$ —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 $\langle 1.14 \rangle$ —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 $\langle 1.02 \rangle$ —Perform the test with 0.25 g of L-Threonine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <1.07>—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) Arsenic $\langle 1.11 \rangle$ —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).

(7) Related substances—Dissolve 0.30 g of L-Threonine in 50 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample 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 these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 5 μ L each of the sample solution and 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 dry the plate at 80°C for 30 minutes. Spray evenly the plate with a solution of ninhydrin in acetone (1 in 50), and heat the plate at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.20% (1 g, 105°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = $11.91 \text{ mg of } C_4H_9NO_3$

Containers and storage Containers—Tight containers.

Thrombin

トロンビン

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.

It contains not less than 80% and not more than 150% of the labeled Units of thrombin.

Each mg contains not less than 10 Units of thrombin.

Description Thrombin is a white to light yellow, amor-

JP XVI

phous substance.

Thrombin (500 Units) dissolves in 1.0 mL of isotonic sodium chloride solution clearly or with slight turbidity within 1 minute.

Loss on drying <2.41> Not more than 3% (50 mg, in vacuum, phosphorus (V) oxide, 4 hours).

Sterility <4.06> It meets the requirement.

Assay (i) Fibrinogen solution—Weigh accurately about 30 mg of fibrinogen, and dissolve in 3 mL of isotonic sodium chloride solution. 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 percentage of the clot in the fibrinogen. Dissolve the fibrinogen in isotonic sodium chloride solution 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 disodium hydrogen-phosphate TS), and dilute with isotonic sodium chloride solution to make a 0.10% solution.

(ii) Procedure—Dissolve Thrombin RS in isotonic sodium chloride solution, and prepare four kinds of standard solutions which contain 4.0, 5.0, 6.2, and 7.5 Units in 1 mL. Transfer accurately 0.10 mL each of the standard solutions maintained at a given degree $\pm 1^{\circ}$ C between 20°C and 30°C to a small test tube, 10 mm in inside 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 kinds of 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 solution 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 five times, determine the clotting times, and calculate the average value. Plot the average values of the clotting times of the four kinds of the 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 sample solution.

Units of 1 container of Thrombin = $U \times 10 \times V$

V: The number of mL of the volume in which the contents of 1 container of Thrombin has been dissolved

Calculate the units for 1 mg of the contents.

Containers and storage Containers—Hermetic containers. Storage—Not exceeding 10°C.

Expiration date 36 months after preparation.

Thymol





C₁₀H₁₄O: 150.22 5-Methyl-2-(1-methylethyl)phenol [89-83-8]

Thymol contains not less than 98.0% of $C_{10}H_{14}O$.

Description Thymol occurs as colorless crystals or white, crystalline masses. It has an aromatic odor, and has a burning taste.

It is very soluble in acetic acid (100), freely soluble in ethanol (95) and in diethyl ether, and slightly soluble in water.

It 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 develops by reflected light and a red-purple color develops by transmitted light.

(2) Dissolve 1 g of Thymol in 5 mL of a solution of sodium hydroxide (1 in 10) by heating in a water bath, and continue heating for several minutes: a light yellow-red color slowly develops. 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 gradually develops.

(3) Triturate Thymol with an equal mass of camphor or menthol: the mixture liquefies.

Melting point <2.60> 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 may develop, but no blue to purple color develops.

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 $\langle 2.50 \rangle$ 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.

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.

Dried Thyroid

乾燥甲状腺

Dried Thyroid is the fresh thyroid gland, previously deprived of connective tissue and fat, minced, dried rapidly at a temperature not above 50°C, and powdered, or diluted with suitable diluents. It is obtained from domesticated animals that are used for food by man.

It contains not less than 0.30% and not more than 0.35% of iodine (I: 126.90) in the form of organic compounds peculiar to the thyroid gland.

Description Dried Thyroid occurs as a light yellow to grayish brown powder. It has a slight, characteristic, meat-like odor.

Identification Mount Dried Thyroid in diluted formaldehyde solution (1 in 10), stain in hematoxylin TS for 10 to 30 minutes, wash with water, soak in a mixture of 1 mL of hydrochloric acid and 99 mL of diluted ethanol (7 in 10) for 5 to 10 seconds, and again wash with water for about 1 hour. Stain in a solution of eosin Y (1 in 100) for 1 to 5 minutes, wash with water, dehydrate, and soak successively in diluted ethanol (7 in 10) for 5 to 10 seconds, in diluted ethanol (4 in 5) for 5 to 10 seconds, in diluted ethanol (9 in 10) for 1 to 2 minutes, in ethanol (95) for 1 to 5 minutes then in ethanol (99.5) for 1 to 5 minutes. Interpenetrate in xylene, seal with balsam, and examine under a microscope: epithelial nuclei forming follicles peculiar to the thyroid gland are observed.

Purity (1) Inorganic iodides—Mix 1.0 g of Dried Thyroid with 10 mL of a saturated solution of zinc sulfate, shake for 5 minutes, and filter. To 5 mL of the filtrate add 0.5 mL of starch TS, 4 drops of sodium nitrite TS and 4 drops of dilute sulfuric acid with thorough shaking: no blue color is produced.

(2) Fat—Extract 1.0 g of Dried Thyroid with diethyl ether for 2 hours using a Soxhlet extractor. Evaporate the diethyl ether extract, and dry the residue at 105° C to constant mass: the mass of the residue is not more than 30 mg.

Loss on drying $\langle 2.41 \rangle$ Not more than 6.0% (1 g, 105°C, constant mass).

Total ash $\langle 5.01 \rangle$ Not more than 5.0% (0.5 g).

Assay Transfer about 1 g of Dried Thyroid, accurately weighed, to a crucible, add 7 g of potassium carbonate, mix carefully, and gently tap the crucible on the table to compact the mixture. Overlay with 10 g of potassium carbonate, and compact again thoroughly by tapping. Place the crucible in a muffle furnace preheated to a temperature between 600°C and 700°C, and ignite the mixture for 25 minutes. Cool, add 20 mL of water, heat gently to boiling, and filter into a flask. To the residue add 20 mL of water, boil, and filter into the same flask. Rinse the crucible and the char on the funnel with boiling water until the filtrate measures 200 mL. Add slowly 7 mL of freshly prepared bromine TS, 40 mL of diluted phosphoric acid (1 in 2), and boil until starch iodide

paper is no longer colored blue by the evolved gas. Wash down 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 at not less than 200 mL. Cool, add 5 mL of a solution of phenol (1 in 20), again rinse 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 $\langle 2.50 \rangle$ 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 = 0.2115 mg of I

Containers and storage Containers—Tight containers.

Tiapride Hydrochloride

チアプリド塩酸塩



C₁₅H₂₄N₂O₄S.HCl: 364.89 *N*-[2-(Diethylamino)ethyl]-2-methoxy-5-(methylsulfonyl)benzamide monohydrochloride [*51012-33-0*]

Tiapride Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of $C_{15}H_{24}N_2O_4S.HCl.$

Description Tiapride Hydrochloride occurs as a white to slightly yellowish white crystal or crystalline powder.

It is very soluble in water, freely soluble in acetic acid (100), soluble in methanol, slightly soluble in ethanol (99.5) and very slightly soluble in acetic anhydride.

It dissolves in 0.1 mol/L hydrochloric acid TS.

Identification (1) Determine the absorption spectrum of a solution of Tiapride Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tiapride Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Tiapride Hydrochloride (1 in 20) responds to Qualitative Tests $\langle 1.09 \rangle$ for chloride.

Purity (1) Heavy metals $\langle 1.07 \rangle$ —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).

(2) Related substances—Dissolve 0.20 g of Tiapride Hydrochloride in 10 mL of methanol and use this solution as the sample solution. Pipet 1 mL of the sample 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 these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot rapidly 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography under a stream of nitrogen. Develop the plate with a mixture of water, 1-butanol and acetic acid (100) (2:2:1) to a distance of about 10 cm, and air-dry, and then dry the plate at 80°C for 30 minutes. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution.

(3) Residual solvent—Being specified separately.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Tiapride Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

> Each mL of 0.1 mol/L perchloric acid VS = 36.49 mg of $C_{15}H_{24}N_2O_4S.HCl$

Containers and storage Containers-Well-closed containers.

Tiapride Hydrochloride Tablets

チアプリド塩酸塩錠

Tiapride Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of tiapride ($C_{15}H_{24}N_2O_4S$: 328.43).

Method of preparation Prepare as directed under Tablets, with Tiapride Hydrochloride.

Identification To a quantity of powdered Tiapride Hydrochloride Tablets, equivalent to 10 mg of tiapride $(C_{15}H_{24}N_2O_4S)$ according to the labeled amount, add 100 mL of 0.1 mol/L hydrochloric acid TS, shake well, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: it exhibits a maximum between 286 nm and 290 nm.

Uniformity of dosage units $\langle 6.02 \rangle$ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Tiapride Hydrochloride Tablets add V/10 mL of 0.1 mol/L hydrochloric acid TS, treat with ultrasonic waves until the tablet is disintegrated, and add 4V/10 mL of methanol. To this solution add exactly V/10 mL of the internal standard solution, shake for 30 minutes, and add methanol to make V mL so that each mL contains about 1 mg of tiapride (C₁₅H₂₄N₂O₄S). Centrifuge this solution for 10 minutes, and use the supernatant liquid as the sample solution. Proceed as directed in the Assay.

Amount (mg) of tiapride ($C_{15}H_{24}N_2O_4S$) = $M_S \times Q_T/Q_S \times V/100 \times 0.900$

 $M_{\rm S}$: Amount (mg) of tiapride hydrochloride for assay

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (1 in 500).

Dissolution Being specified separately.

Assay Weigh accurately the mass of not less than 20 Tiapride Hydrochloride Tablets and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of tiapride (C15H24N2O4S), add about 10 mL of 0.1 mol/L hydrochloric acid TS and 40 mL of methanol, add exactly 10 mL of the internal standard solution, shake for 30 minutes, and add methanol to make 100 mL. Centrifuge this solution and use the supernatant liquid as the sample solution. Separately, weigh accurately about 0.11 g of tiapride chloride for assay, previously dried at 105°C for 2 hours, dissolve in 10 mL of 0.1 mol/L hydrochloric acid TS, add exactly 10 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the standard solution. Perform the test with 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of Tiapride to that of the internal standard.

Amount (mg) of tiapride (C₁₅H₂₄N₂O₄S)
=
$$M_{\rm S} \times Q_{\rm T}/Q_{\rm S} \times 0.900$$

 $M_{\rm S}$: Amount (mg) of tiapride chloride for assay

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (1 in 500).

Operating conditions—

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

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

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

Mobile phase: Dissolve 11.2 g of sodium perchlorate in 800 mL of water, add 5 mL of diluted perchloric acid (17 in 2000). To 800 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of tiapride is about 8 minutes.

System suitability-

System performance: When the procedure is run with $5 \,\mu L$ of the standard solution under the above operating conditions, tiapride and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

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

Containers and storage Containers-Well-closed containers.

Tiaramide Hydrochloride

チアラミド塩酸塩



C₁₅H₁₈ClN₃O₃S.HCl: 392.30 5-Chloro-3-{2-[4-(2-hydroxyethyl)piperazin-1-yl]-2-oxoethyl}-1,3-benzothiazol-2(3*H*)-one monohydrochloride [*35941-71-0*]

Tiaramide Hydrochloride, when dried, contains not less than 98.5% of $C_{15}H_{18}ClN_3O_3S.HCl.$

Description Tiaramide Hydrochloride occurs as a white, crystalline powder. It is odorless.

It is freely soluble in water, slightly soluble in ethanol (95) and in acetic acid (100), and practically insoluble in acetic anhydride and in diethyl ether.

The pH of a solution of Tiaramide Hydrochloride (1 in 20) is between 3.0 and 4.5.

Melting point: about 265°C (with decomposition).

Identification (1) Dissolve 5 mg of Tiaramide Hydrochloride in 5 mL of 0.1 mol/L hydrochloric acid TS, and add 3 drops of Dragendorff's TS: an orange precipitate is formed.

(2) Determine the infrared absorption spectrum of Tiaramide Hydrochloride, previously dried, as directed in the potassium chloride disk method under the Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Tiaramide Hydrochloride (1 in 50) responds to the Qualitative Tests $\langle 1.09 \rangle$ for chloride.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Tiaramide Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals $\langle 1.07 \rangle$ —Proceed with 2.0 g of Tiaramide 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 $\langle 1.11 \rangle$ —Prepare the test solution with 1.0 g of Tiaramide Hydrochloride according to Method 1, and perform the test. In the procedure, add 20 mL of diluted hydrochloric acid (1 in 2) (not more than 2 ppm).

(4) Related substances—Dissolve 0.20 g of Tiaramide Hydrochloride in 10 mL of diluted ethanol (7 in 10), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add diluted ethanol (7 in 10) to make exactly 100 mL. Pipet 2 mL of this solution, add diluted ethanol (7 in 10) 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 <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thinlayer chromatography. After air-drying, immediately develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, air-dry the plate, and then dry at 100°C for 30 minutes. After cooling, examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and the spot of the starting point from the sample solution are not more intense than the spot from the standard solution. Allow the plate to stand in iodine vapor for 30 minutes: the spots other than the principal spot and the spot of the starting point from the sample solution are not more intense than the spot from the spot of the starting point from the sample solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Tiaramide Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3) by warming, cool, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS until the color of the solution changes from red through purple to blue-purple (indicator: 3 drops of neutral red TS). Perform a blank determination, and make any necessary correction.

> Each mL of 0.1 mol/L perchloric acid VS = $39.23 \text{ mg of } C_{15}H_{18}ClN_3O_3S.HCl$

Containers and storage Containers—Well-closed containers.

Tiaramide Hydrochloride Tablets

チアラミド塩酸塩錠

Tiaramide Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of tiaramide ($C_{15}H_{18}CIN_3O_3S$: 355.84).

Method of preparation Prepare as directed under Tablets, with Tiaramide Hydrochloride.

Identification (1) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: it exhibits maxima between 285 nm and 289 nm, and between 292 nm and 296 nm.

(2) To a quantity of powdered Tiaramide Hydrochloride Tablets, equivalent to 0.1 g of tiaramide according to the labeled amount, add 10 mL of diluted ethanol (7 in 10), shake well, filter, and use the filtrate as the sample solution. Separately, dissolve 0.11 g of tiaramide hydrochloride for assay in 10 mL of diluted ethanol (7 in 10), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop with a mixture of 1-butamol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, and dry the plate at 100°C for 30 minutes. Spray evenly Dragendorff's TS for spraying followed by diluted nitric acid (1 in 50) on the plate: the principal spot obtained with the sample solution and the spot with the standard solution are yellow-red in color and have the same Rf value.

Uniformity of dosage units <6.02> Perform the test accord-

ing to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Tiaramide Hydrochloride Tablets add a volume of 0.1 mol/L hydrochloric acid TS, equivalent to 3/5 volume of V mL which makes a solution so that each mL contains about 1 mg of tiaramide (C15H18ClN3O3S), shake for 60 minutes, then add 0.1 mol/L hydrochloric acid TS to make exactly VmL, and filter. Discard the first 20 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 55 mg of tiaramide hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 50 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, $A_{\rm T}$ and $A_{\rm S}$, of the sample solution and standard solution at 294 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

> Amount (mg) of tiaramide (C₁₅H₁₈ClN₃O₃S) = $M_{\rm S} \times A_{\rm T}/A_{\rm S} \times V/50 \times 0.907$

 $M_{\rm S}$: Amount (mg) of tiaramide hydrochloride for assay

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rates of a 50-mg tablet in 15 minutes and of a 100-mg tablet in 30 minutes are not less than 80%.

Start the test with 1 tablet of Tiaramide Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding $0.5 \,\mu\text{m}$. 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 56 μ g of tiaramide (C₁₅H₁₈ClN₃O₃S) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 15 mg of tiaramide hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 25 mL, and use this solution as the standard solution. Determine the absorbances, $A_{\rm T}$ and $A_{\rm S}$, at 294 nm of the sample solution and standard solution as directed under Ultravioletvisible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of tiaramide ($C_{15}H_{18}ClN_3O_3S$)

 $= M_{\rm S} \times A_{\rm T}/A_{\rm S} \times V'/V \times 1/C \times 360 \times 0.907$

 $M_{\rm S}$: Amount (mg) of tiaramide hydrochloride for assay

C: Labeled amount (mg) of tiaramide ($C_{15}H_{18}ClN_3O_3S$) in 1 tablet

Assay Weigh accurately the mass of more than 20 Tiaramide Hydrochloride Tablets, and powder. Weigh accurately an amount of the powder, equivalent to about 0.1 g of tiaramide ($C_{15}H_{18}ClN_3O_3S$), add 60 mL of 0.1 mol/L hydrochloric acid TS, shake for 30 minutes, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.11 g of tiaramide hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in 0.1 mol/L hydrochloric acid TS 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 sample solution and standard solution at 294 nm as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$.

Amount (mg) of tiaramide (
$$C_{15}H_{18}ClN_3O_3S$$
)
= $M_S \times A_T/A_S \times 0.907$

 $M_{\rm S}$: Amount (mg) of tiaramide hydrochloride for assay

Containers and storage Containers-Tight containers.

Ticlopidine Hydrochloride





C₁₄H₁₄ClNS.HCl: 300.25 5-(2-Chlorobenzyl)-4,5,6,7tetrahydrothieno[3,2-*c*]pyridine monohydrochloride [*53885-35-1*]

Ticlopidine Hydrochloride contains not less than 99.0% of $C_{14}H_{14}CINS.HCl$, calculated on the anhydrous basis.

Description Ticlopidine Hydrochloride occurs as a white to pale yellowish white crystalline powder.

It is freely soluble in acetic acid (100), soluble in water and in methanol, sparingly soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification (1) Determine the infrared absorption spectrum of Ticlopidine Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Ticlopidine Hydrochloride (1 in 20) responds to the Qualitative Tests $\langle 1.09 \rangle$ (2) for chloride.

Purity (1) Heavy metals $\langle 1.07 \rangle$ —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 $\langle 1.11 \rangle$ —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 a solution of hydrochloric acid in methanol (1 in 20,000), and use this solution as the sample solution. To exactly 5 mL of the sample solution add a solution of hydrochloric acid in methanol (1 in 20,000) to make exactly 200 mL, and use this solution as the standard solution (1). Separately, pipet 1 mL of the sample solution, add a solution of hydrochloric acid in methanol (1 in 20,000) to make exactly 50 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 10 μ L each of the sample solution and standard solution (1) on a plate of silica gel for thin-layer chromatography (Plate 1), and

spot 10 μ L each of the sample solution and standard solution (2) on another plate of silica gel 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 the principal spot from the standard solution (1). Allow Plate 2 to stand in an iodine vapor for 30 minutes: the spots other than the principal spot from the sample solution are not more intense than the principal spot from the sample 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. To 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 exactly 0.54 g of formaldehyde solution, and add water to make exactly 1000 mL. To exactly 10 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 $\langle 2.48 \rangle$ Not more than 1.0% (0.3 g, volumetric titration, direct titration).

Residue on ignition $\langle 2.44 \rangle$ 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 $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

> Each mL of 0.1 mol/L perchloric acid VS = $30.03 \text{ mg of } C_{14}H_{14}CINS.HCl$

Containers and storage Containers-Well-closed containers.

Timepidium Bromide Hydrate

チメピジウム臭化物水和物



 $C_{17}H_{22}BrNOS_2.H_2O:$ 418.41 (*5RS*)-3-(Dithien-2-ylmethylene)-5-methoxy-1,1dimethylpiperidinium bromide monohydrate [*35035-05-3*, anhydride]

Timepidium Bromide Hydrate contains not less than 98.5% of timepidium bromide ($C_{17}H_{22}BrNOS_2$: 400.40), calculated on the anhydrous basis.

Description Timepidium Bromide Hydrate occurs as white crystals or crystalline powder.

It is very soluble in methanol and in acetic acid (100), freely soluble in ethanol (99.5), sparingly soluble in water and in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Timepidium Bromide Hydrate in freshly boiled and cooled water (1 in 100) is between 5.3 and 6.3.

A solution of Timepidium Bromide Hydrate in methanol (1 in 20) shows no optical rotation.

Identification (1) To 1 mL of a solution of Timepidium Bromide Hydrate (1 in 100) add 1 mL of ninhydrin-sulfuric acid TS: a red purple color develops.

(2) Determine the absorption spectrum of a solution of Timepidium Bromide Hydrate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Timepidium Bromide Hydrate as directed in the potassium bromide disk method under the Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhitit similar intensities of absorption at the same wave numbers.

(4) A solution of Timepidium Bromide Hydrate (1 in 100) responds to the Qualitative Tests $\langle 1.09 \rangle$ (1) for Bromide.

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

(2) Heavy metals $\langle 1.07 \rangle$ —Proceed with 1.0 g of Timepidium Bromide 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).

(3) Related substances—Dissolve 0.10 g of Timepidium Bromide Hydrate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample 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 these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, water, acetic acid (100) and ethyl acetate (5:4:1:1:1) to a distance of about 13 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water $\langle 2.48 \rangle$ 3.5 – 5.0% (0.4 g, volumetric titration, direct titration).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Timepidium Bromide Hydrate, dissolve in 60 mL of a mixture of acetic anhydride and acetic acid (100) (2:1), and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 40.04 mg of $C_{17}H_{22}BrNOS_2$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Timolol Maleate

チモロールマレイン酸塩



 $C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4: 432.49$ (2S)-1-[(1,1-Dimethylethyl)amino]-3-(4-morpholin-4-yl-1,2,5-thiadiazol-3-yloxy)propan-2-ol monomaleate
[26921-17-5]

Timolol Maleate, when dried, contains not less than 98.0% and not more than 101.0% of $C_{13}H_{24}N_4O_3S$. $C_4H_4O_4$.

Description Timolol Maleate occurs as a white to pale yellowish white crystalline powder.

It is freely soluble in acetic acid (100), and soluble in water and in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS.

Melting point: about 197°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Timolol Maleate in 0.1 mol/L hydrochloric acid TS (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Timolol Maleate as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(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.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_D^{20}$: $-5.7 - -6.2^\circ$ (after drying, 1.25 g, 1 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH $\langle 2.54 \rangle$ The pH of a solution prepared by dissolving 1.0 g of Timolol 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 $\langle 2.24 \rangle$, is not more than 0.05.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Timolol Maleate 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) Related substances—Dissolve 30 mg of Timolol

Maleate in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 25 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> 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 obtained from sample solution is not larger than 1/5 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 1/2 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 inside diameter and 25 cm in length, packed with phenylsilanized silica gel for liquid chromatography (5 μ m 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, adjust 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.

Time span of measurement: About 2 times as long as the retention time of timolol beginning after the solvent peak. *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 $25 \,\mu$ L of this solution is equivalent to 7 to 13% of that from $25 \,\mu$ L of the standard solution.

System performance: When the procedure is run with 25 μ L of the sample solution under the above operating conditions, 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 $25 \,\mu\text{L}$ 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%.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, in vacuum, 100°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). 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 C₁₃H₂₄N₄O₃S.C₄H₄O₄

Containers and storage Containers—Tight containers.

Tinidazole

チニダゾール



 $C_8H_{13}N_3O_4S: 247.27$ 1-[2-(Ethylsulfonyl)ethyl]-2-methyl-5-nitro-1*H*-imidazole

[19387-91-8]

Tinidazole, when dried, contains not less than 98.5% and not more than 101.0% of $C_8H_{13}N_3O_4S$.

Description Tinidazole occurs as a light yellow, crystalline powder.

It is soluble in acetic anhydride and in acetone, sparingly soluble in methanol, slightly soluble in ethanol (99.5), and very slightly soluble in water.

Identification (1) Determine the absorption spectrum of a solution of Tinidazole in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tinidazole as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 125 – 129°C

Purity (1) Sulfate $\langle 1.14 \rangle$ —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 <1.07>—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 $\langle 1.11 \rangle$ —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 2 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone 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 $\langle 2.03 \rangle$. Spot 10 μ L each of the sample 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 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 sample solution are not more intense than the spot from the standard solution.

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Loss on drying $\langle 2.41 \rangle$ Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 24.73 mg of $C_8H_{13}N_3O_4S$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Tipepidine Hibenzate



C₁₅H₁₇NS₂.C₁₄H₁₀O₄: 517.66 3-(Dithien-2-ylmethylene)-1-methylpiperidine mono[2-(4hydroxybenzoyl)benzoate] [*31139-87-4*]

Tipepidine Hibenzate, when dried, contains not less than 98.5% of $C_{15}H_{17}NS_2.C_{14}H_{10}O_4$.

Description Tipepidine Hibenzate occurs as a white to light yellow, crystalline powder. It is odorless and tasteless.

It is freely soluble in acetic acid (100), slightly soluble in methanol and in ethanol (95), very slightly soluble in water, and practically insoluble in diethyl ether.

Identification (1) Dissolve 0.01 g of Tipepidine Hibenzate in 5 mL of sulfuric acid: an orange-red color develops.

(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 portions 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 light red precipitate is formed.

(3) Determine the absorption spectrum of a solution of Tipepidine Hibenzate in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum; both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Tipepidine Hibenzate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 189 – 193°C

Purity (1) Clarity 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 $\langle 2.24 \rangle$: its absorbance at 400 nm is not more than 0.16.

(2) Heavy metals $\langle 1.07 \rangle$ —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 $\langle 1.11 \rangle$ —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 sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of peaks other than hibenzic acid and tipepidine from the sample 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 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 50°C.

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 about 12 minutes.

Time span of measurement: As long as the retention time of tipepidine beginning after the solvent peak.

System suitability—

Test for required detection: 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 μ L of this solution is equivalent to 7 to 13% of that from 20 μ L 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 $20 \,\mu$ L of this solution under the above operating conditions, hibenzic acid, tipepidine and propyl parahydroxybenzoate are eluted in this order with the resolution between the peaks of tipepidine and propyl parahydroxybenzoate being not less than 3.

System repeatability: When the test is repeated 6 times with $20 \,\mu\text{L}$ 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%.

(ii) Dissolve 10 mg of Tipepidine Hibenzate in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the au-

tomatic integration method: the total area of peaks other than hibenzic acid and tipepidine from the sample solution is not larger than 1/2 times the peak area of the tipepidine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-length: 254 nm).

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

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

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 about 10 minutes.

Time span of measurement: As long as the retention time of tipepidine beginning after the solvent peak.

System suitability-

Test for required detection: 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 μ L of this solution is equivalent to 7 to 13% of that from 20 μ L of the standard solution.

System performance: Dissolve 12 mg of Tipepidine Hibenzate and 4 mg of xanthene in 50 mL of the mobile phase. When the procedure is run with $10 \,\mu$ L of this solution under the above operating conditions, hibenzic acid, tipepidine and xanthene are eluted in this order with the resolution between the peaks of tipepidine and xanthene being not less than 3.

System repeatability: When the test is repeated 6 times with $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tipepidine is not more than 3.0%.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 60°C, in vacuum, phosphorus (V) oxide, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

> Each ml of 0.1 mol/L perchloric acid VS = 51.77 mg of C₁₅H₁₇NS₂.C₁₄H₁₀O₄

Containers and storage Containers— Well-closed containers.

Storagle—Light-resistant.

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Tipepidine Hibenzate Tablets

チペピジンヒベンズ酸塩錠

Tipepidine Hibenzate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of tipepidine hibenzate ($C_{15}H_{17}NS_2.C_{14}H_{10}O_4$: 517.66).

Method of preparation Prepare as directed under Tablets, with Tipepidine Hibenzate.

Identification (1) To a quantity 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 portions 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 light red precipitate is formed.

(2) To a quantity 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 $\langle 2.24 \rangle$: it exhibits a maximum between 282 nm and 286 nm.

Uniformity of dosage units $\langle 6.02 \rangle$ 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) (1 in 2) and 15 mL of methanol per 11 mg of tipepidine hibenzate ($C_{15}H_{17}NS_2.C_{14}H_{10}O_4$), and warm for 15 minutes with occasional shaking. After cooling, add diluted methanol (1 in 2) to make exactly *V* mL so that each mL contains about 0.44 mg of tipepidine hibenzate ($C_{15}H_{17}NS_2.C_{14}H_{10}O_4$), and filter. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution, then add diluted methanol (1 in 2) to make 25 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of tipepidine hibenzate ($C_{15}H_{17}NS_2.C_{14}H_{10}O_4$) = $M_S \times Q_T/Q_S \times V/50$

 $M_{\rm S}$: Amount (mg) of tipepidine hibenzate for assay

Internal standard solution—A solution of dibucaine hydrochloride in methanol (1 in 2000).

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Tipepidine Hibenzate Tablets is not less than 80%.

Start the test with 1 tablet of Tipepidine Hibenzate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, filter, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 0.11 g

of tipepidine hibenzate for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 60°C) for 3 hours, and dissolve in 80 mL of diluted ethanol (3 in 4) by warming occasionally. After cooling, add diluted ethanol (3 in 4) to make exactly 100 mL, then pipet 20 mL of this solution, add water to make exactly 900 mL, and use this solution 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 sample solution and standard solution as directed under Ultravioletvisible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of tipepidine hibenzate ($C_{15}H_{17}NS_2.C_{14}H_{10}O_4$) = $M_S \times (A_{T1} - A_{T2}/A_{S1} - A_{S2}) \times 1/C \times 20$

 $M_{\rm S}$: Amount (mg) of tipepidine hibenzate for assay C: Labeled amount (mg) of tipepidine hibenzate $(C_{15}H_{17}NS_2.C_{14}H_{10}O_4)$ in 1 tablet

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 (C₁₅H₁₇NS₂.C₁₄H₁₀O₄), 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 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution, then add diluted methanol (1 in 2) to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of tipepidine hibenzate for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 60°C) for 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 mL of this solution, 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 standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of tipepidine to that of the internal standard, respectively.

Amount (mg) of tipepidine hibenzate (C₁₅H₁₇NS₂.C₁₄H₁₀O₄) = $M_{\rm S} \times Q_{\rm T}/Q_{\rm S}$

 $M_{\rm S}$: Amount (mg) of tipepidine hibenzate for assay

Internal standard solution—A solution of dibucaine hydrochloride in methanol (1 in 2000).

Operating conditions—

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

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

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

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 suitability-

System performance: When the procedure is run with 20

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 μ L 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.

System repeatability: When the test is repeated 6 times with $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of tipepidine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Titanium Oxide

酸化チタン

TiO₂: 79.87

Titanium Oxide, when dried, contains not less than 98.5% of TiO₂.

Description Titanium Oxide occurs as a white powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (99.5) and in diethyl ether.

It dissolves in hot sulfuric acid and in hydrofluoric acid, and does not dissolve in hydrochloric acid, in nitric acid and in dilute sulfuric acid.

When fused by heating with potassium hydrogen sulfate, with potassium hydroxide, or with potassium carbonate, it changes to soluble salts.

Shake 1 g of Titanium Oxide with 10 mL of water: the mixture is neutral.

Identification Heat 0.5 g of Titanium Oxide with 5 mL of sulfuric acid until white fumes are evolved, cool, add cautiously water to make 100 mL, and filter. To 5 mL of the filtrate add 2 to 3 drops of hydrogen peroxide TS: a yellow-red color develops.

Purity (1) Lead—Place 1.0 g of Titanium Oxide in a platinum crucible, add 10.0 g of potassium hydrogen sulfate, heat gently with caution at the beginning, then raise the temperature gradually, and heat strongly with occasional shaking until the contents fuse to yield a clear liquid. Cool, add 30 mL of a solution of diammonium hydrogen citrate (9 in 20) and 50 mL of water, dissolve by heating on a water bath, cool, add water to make 100 mL, and use this solution as the sample stock solution. Take 25 mL of the sample stock solution to a separator, add 10 mL of a solution of ammonium sulfate (2 in 5) and 5 drops of thymol blue TS, neutralize with ammonia TS, and add 2.5 mL of ammonia TS. To this solution add exactly 20 mL of a solution of dithizone in nbutyl acetate (1 in 500), shake for 10 minutes, and use this nbutyl acetate solution as the sample solution. Separately, place 6.0 mL of Standard Lead Solution in a platinum crucible, proceed as directed in the sample solution, and use this solution as the standard solution. Determine the absorbances of the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions: the absorbance of the sample solution is smaller than that of the standard solution (not more than 60 ppm).

Gas: Combustible gas—Acetylene gas or hydrogen gas. Supporting gas—Air.

Lamp: Lead hollow-cathode lamp.

(2) Arsenic $\langle 1.11 \rangle$ —Perform the test with 20 mL of the sample stock solution obtained in (1) as the test solution: the color is not deeper than the following color standard.

Color standard: Proceed in the same manner without Titanium Oxide, transfer 20 mL of the obtained solution to a generator bottle, add 2.0 mL of Standard Arsenic Solution, and proceed in the same manner as the test with the test solution (not more than 10 ppm).

(3) Water-soluble substances—Shake thoroughly 4.0 g of Titanium Oxide with 50 mL of water, and allow to stand overnight. Shake thoroughly with 2 mL of ammonium chloride TS, add further 2 mL of ammonium chloride TS if necessary, and allow titanium oxide to settle. Add water to make 200 mL, shake thoroughly, and filter through double filter paper. Discard the first 10 mL of the filtrate, evaporate 100 mL of the clear filtrate on a water bath, and heat strongly at 650°C to constant mass: the mass of the residue is not more than 5.0 mg.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 3 hours).

Assay Weigh accurately about 0.2 g of Titanium Oxide, previously dried, transfer to a crucible, and add 3 g of potassium disulfate. Cover, and heat gently at first, gradually raise the temperature, and then heat the fused contents for 30 minutes. Continue heating for 30 minutes at a higher temperature to make the fused mixture a deep yellow-red, almost clear liquid. Cool, transfer the contents of the crucible to a 250-mL beaker, wash the crucible with a mixture of 75 mL of water and 2.5 mL of sulfuric acid into the beaker, and heat on a water bath until the solution becomes almost clear. Dissolve 2 g of L-tartaric acid in the solution, add 2 to 3 drops of bromothymol blue TS, neutralize with ammonia TS, and acidify with 1 to 2 mL of diluted sulfuric acid (1 in 2). Pass hydrogen sulfide sufficiently through the solution, add 30 mL of ammonia TS, again saturate the solution with hydrogen sulfide, allow to stand for 10 minutes, and filter. Wash the precipitate on the filter paper with ten 25-mL portions of a mixture of ammonium L-tartrate solution (1 in 100) and ammonium sulfide TS (9:1). When the precipitate is filtered and washed, prevent iron (II) sulfide from oxidation by filling the solution on the filter paper. Combine the filtrate and the washings, add 40 mL of diluted sulfuric acid (1 in 2), and boil to expel hydrogen sulfide. Cool, and dilute with water to make 400 mL. Add gradually 40 mL of cupferron TS to the solution with stirring, and allow to stand. After sedimentation of a yellow precipitate, add again cupferron TS until a white precipitate is produced. Filter by slight suction using quantitative filter paper, wash with twenty portions of diluted hydrochloric acid (1 in 10), and remove water by stronger suction at the last washing. Dry the precipitate together with the filter paper at 70°C, transfer to a tared crucible, and heat very gently at first, and raise the temperature gradually after smoke stops evolving. Heat strongly between 900°C and 950°C to constant mass, cool, and weigh as titanium oxide (TiO₂).

Containers and storage Containers-Well-closed contain-

Wavelength: 283.3 nm.

ers.

Tizanidine Hydrochloride

チザニジン塩酸塩



 $C_9H_8ClN_5S.HCl: 290.17$ 5-Chloro-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-2,1,3-benzothiadiazole-4-amine monohydrochloride [64461-82-1]

Tizanidine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of $C_9H_8CIN_5S.HCl.$

Description Tizanidine Hydrochloride occurs as a white to light yellowish white crystalline powder.

It is soluble in water, slightly soluble in ethanol (99.5), and practically insoluble in acetic anhydride and in acetic acid (100).

Melting point: about 290°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Tizanidine Hydrochloride in diluted 1 mol/L ammonia TS (1 in 10) (1 in 125,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tizanidine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Tizanidine Hydrochloride (1 in 50) responds to the Qualitative Tests $\langle 1.09 \rangle$ for chloride.

Purity (1) Heavy metals $\langle 1.07 \rangle$ —Proceed with 1.0 g of Tizanidine Hydrochloride according to Method 3, 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 60 mg of Tizanidine Hydrochloride in 10 mL of a mixture of water and acetonitrile (17:3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of water and acetonitrile (17:3) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than tizanidine with the sample solution is not larger than 1/5 times the peak area of tizanidine with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm for about 3 minutes after sample injection and 318 nm subsequently).

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

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

Mobile phase A: A mixture of water and formic acid (200:1), adjusted to pH 8.5 with ammonia water (28).

Mobile phase B: A mixture of acetonitrile and the mobile phase A (4:1).

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

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 - 10	$81 \rightarrow 68$	$19 \rightarrow 32$
10 - 13 13 - 26	$68 \rightarrow 10$	$32 \rightarrow 90$
26 - 28	10	90

Flow rate: Adjust the flow rate so that the retention time of tizanidine is about 7 minute.

Time span of measurement: About 4 times as long as the retention time of tizanidine beginning after the solvent peak. *System suitability*—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mixture of water and acetonitrile (17:3) to make exactly 10 mL. Confirm that the peak area of tizanidine obtained with 10 μ L of this solution is equivalent to 14 to 26% of that with 10 μ L of the standard solution.

System performance: Dissolve 2 mg each of Tizanidine Hydrochloride and *p*-toluenesulfonic acid monohydrate in 100 mL of the mixture of water and acetonitrile (17:3). When the procedure is run with $10 \,\mu$ L of this solution under the above operating conditions, *p*-toluenesulfonic acid and tizanidine 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 $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tizanidine is not more than 2.0%.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.2% (1 g, 105°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Tizanidine Hydrochloride, previously dried, dissolve in 60 mL of a mixture of acetic anhydride and acetic acid (100) (7:3) with the aid of warming. After cooling, titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

> Each mL of 0.1 mol/L perchloric acid VS = 29.02 mg of C₉H₈ClN₅S.HCl

Containers and storage Containers-Well-closed containers.
JP XVI

Tobramycin

トブラマイシン



C₁₈H₃₇N₅O₉: 467.51 3-Amino-3-deoxy- α -D-glucopyranosyl-(1 \rightarrow 6)-[2,6-diamino-2,3,6-trideoxy- α -D-*ribo*-hexopyranosyl-(1 \rightarrow 4)]-2-deoxy-D-streptamine [*32986-56-4*]

Tobramycin is an aminoglycoside substance having antibacterial activity produced by the growth of *Streptomyces tenebrarius*.

It contains not less than 900 μ g (potency) and not more than 1060 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Tobramycin is expressed as mass (potency) of tobramycin (C₁₈H₃₇N₅O₉).

Description Tobramycin occurs as a white to pale yellowish white powder.

It is very soluble in water, freely soluble in formamide, slightly soluble in methanol, and very slightly soluble in ethanol (95).

It is hygroscopic.

Identification (1) 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 $\langle 2.21 \rangle$, using sodium 3-trimethylsilylpropanesulfonate 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 between δ 2.6 ppm and δ 4.0 ppm, and a multiple signal C between δ 1.0 ppm and δ 2.1 ppm. The ratio of the integrated intensity of these signals, A:B:C, is about 1:8:2.

(2) Dissolve 10 mg each of Tobramycin and Tobramycin RS in 1 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 4 μ L of the sample 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 airdry the plate. Spray evenly ninhydrin TS on the plate, and heat at 100°C for 5 minutes: the *R*f values of the principal spots obtained from the sample solution and the standard solution are the same.

Optical rotation $\langle 2.49 \rangle$ [α]_D²⁰: +138 - +148° (1 g calculated

on the anhydrous basis, water, 25 mL, 100 mm).

pH $\langle 2.54 \rangle$ 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 $\langle 1.07 \rangle$ —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 sample solution. Pipet 1 mL of the sample 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 <2.03>. Spot 5 μ L of the sample 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, then further dry at 110°C for 10 minutes. Immediately spray evenly a mixture of water and sodium hypochlorite TS (4:1) on the plate, air-dry the plate, then spray potassium iodide-starch TS on the plate: the spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Water $\langle 2.48 \rangle$ 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 $\langle 2.44 \rangle$ Not more than 1.0% (0.5 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism-Bacillus subtilis ATCC 6633

(ii) Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Tobramycin RS, equivalent to about 25 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 25 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. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 8 μ g (potency) and 2 μ g (potency), and use these solutions as the high concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Tobramycin, equivalent to about 25 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 25 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains $8 \mu g$ (potency) and $2 \mu g$ (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers-Tight containers.

Tobramycin Injection

トブラマイシン注射液

Tobramycin Injection is an aqueous injection.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$: 467.51).

Method of preparation Prepare as directed under Injections, with Tobramycin.

Description Tobramycin Injection occurs as a colorless or very pale yellow clear liquid.

Identification To a volume of Tobramycin Injection, equivalent to 10 mg (potency) of Tobramycin according to the labeled amount, add water to make 1 mL, and use this solution as the sample solution. Separately, dissolve 10 mg (potency) of Tobramycin RS in 1 mL of water, and use this solution as the standard solution. Then, proceed as directed in the Identification (2) under Tobramycin.

Osmotic pressure ratio Being specified separately.

pH <2.54> 5.0 – 7.0

Bacterial endotoxins <4.01> Less than 0.50 EU/mg (potency).

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism, culture medium, and standard solutions—Proceed as directed in the Assay under Tobramycin.

(ii) Sample solutions—To exactly 5 mL of Tobramycin Injection add 0.1 mol/L phosphate buffer solution, pH 8.0 so that each mL contains 1 mg (potency) of Tobramycin. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 8 μ g (potency) and 2 μ g (potency), and use these solutions as the concentration sample solution high and the low concentration sample solution, respectively.

Containers and storage Containers—Hermetic containers.

Tocopherol

Vitamin E *dl-α-*Tocopherol





C₂₉H₅₀O₂: 430.71 2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-ol [*10191-41-0*]

To copherol contains not less than 96.0% and not more than 102.0% of $C_{29}H_{50}O_2$.

Description Tocopherol is a clear, yellow to red-brown, viscous liquid. It is odorless.

It is miscible with ethanol (99.5), with acetone, with chloroform, with diethyl ether and with vegetable oils.

It is freely soluble in ethanol (95), and practically insoluble in water.

It is optically inactive.

It is oxidized by air and light, and acquires a dark red color.

Identification (1) Dissolve 0.01 g 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 develops.

(2) Determine the infrared absorption spectrum of Tocopherol as directed in the liquid film method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Tocopherol RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance $\langle 2.24 \rangle = E_{1 \text{ cm}}^{1\%}$ (292 nm): 71.0 – 76.0 (10 mg, ethanol (99.5), 200 mL).

Refractive index <2.45> $n_{\rm D}^{20}$: 1.503 – 1.507

Specific gravity $\langle 2.56 \rangle$ d_{20}^{20} : 0.947 – 0.955

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 Matching Fluid C.

(2) Heavy metals $\langle 1.07 \rangle$ —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 sample solution and standard solution. Perform the test with exactly 20 μ L each of these solutions as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and determine the peak heights, $H_{\rm T}$ and $H_{\rm S}$, of tocopherol in the sample solution and standard solution.

Amount (mg) of $C_{29}H_{50}O_2 = M_S \times H_T/H_S$

M_S: Amount (mg) of Tocopherol RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 292 nm).

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

Column temperature: A constant temperature of about $35^{\circ}C$.

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 0.05 g each of Tocopherol and tocopherol acetate in 50 mL of ethanol (99.5). When the procedure is run with $20 \,\mu$ L 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 $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak heights 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



C31H52O3: 472.74

2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yl acetate [7695-91-2]

Tocopherol Acetate contains not less than 96.0% and not more than 102.0% of $C_{31}H_{52}O_3$.

Description Tocopherol Acetate is a clear, colorless or yellow, viscous and odorless liquid.

It is miscible with ethanol (99.5), with acetone, with chloroform, with diethyl ether, with hexane and with fixed oils.

It is freely soluble in ethanol (95), and practically insoluble in water.

It is optically inactive.

It is affected by air and light.

Identification (1) Dissolve 0.05 g 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 produced.

(2) Determine the infrared absorption spectrum of

Official Monographs / Tocopherol Acetate 1507

Tocopherol Acetate as directed in the liquid film method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Tocopherol Acetate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance <2.24> $E_{1 \text{ cm}}^{1\%}$ (284 nm): 41.0 – 45.0 (10 mg, ethanol (99.5), 100 mL).

Refractive index <2.45> $n_{\rm D}^{20}$: 1.494 – 1.499

Specific gravity $\langle 2.56 \rangle$ d_{20}^{20} : 0.952 – 0.966

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 CS add 0.5 mol/L hydrochloric acid TS to make 100 mL.

(2) Heavy metals $\langle 1.07 \rangle$ —Carbonize 1.0 g of Tocopherol Acetate 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 (20 ppm).

(3) α -Tocopherol—Dissolve 0.10 g of Tocopherol Acetate in exactly 10 mL of hexane, and use this solution as the sample solution. Separately, dissolve 50 mg of Tocopherol RS in hexane to make exactly 100 mL. Pipet 1 mL of this solution, add hexane 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 <2.03>. Spot $10 \,\mu\text{L}$ each of the sample solution and 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 α , α' -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 sample solution corresponding to that from the standard solution is not larger 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 sample solution and standard solution. Perform the test with exactly 20 μ L each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak heights, $H_{\rm T}$ and $H_{\rm S}$, of tocopherol acetate in the sample solution and the standard solution, respectively.

Amount (mg) of $C_{31}H_{52}O_3 = M_S \times H_T/H_S$

 $M_{\rm S}$: Amount (mg) of Tocopherol Acetate RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 284 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel (5 μ m 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 0.05 g each of Tocopherol Acetate and tocopherol in 50 mL of ethanol (99.5). When the procedure is run with $20 \,\mu$ L 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 $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak heights 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



 $C_{66}H_{106}CaO_{10}: 1099.62$ Monocalcium bis{3-[2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yloxycarbonyl]propanoate} [14638-18-7]

To copherol Calcium Succinate, when dried, contains not less than 96.0% and not more than 102.0% of $C_{66}H_{106}CaO_{10}.$

Description Tocopherol Calcium Succinate occurs as a white to yellowish white powder. It is odorless.

It is freely soluble in chloroform and in carbon tetrachloride, and practically insoluble in water, in ethanol (95) and 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.

It dissolves in acetic acid (100).

It is optically inactive.

Identification (1) Dissolve 0.05 g of Tocopherol Calcium Succinate in 1 mL of aetic 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° C for 15 minutes: a red to orange color develops.

(2) Dissolve 0.08 g of Tocopherol Calcium Succinate, previously dried, in 0.2 mL of carbon tetrachloride. Determine the infrared absorption spectrum of the solution as directed in the liquid film method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(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 $\langle 1.09 \rangle$ for calcium salt.

Absorbance $\langle 2.24 \rangle = E_{1 \text{ cm}}^{1\%}$ (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 CS add 0.5 mol/L hydrochloric acid TS to make 100 mL.

(2) Alkalinity—To 0.20 g of Tocopherol Calcium Succinate add 10 mL of diethyl 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 develops in the water layer.

(3) Chloride $\langle 1.03 \rangle$ —Dissolve 0.10 g of Tocopherol Calcium Succinate in 4 mL of acetic acid (100), add 20 mL of water and 50 mL of diethyl ether, shake thoroughly, and collect the water layer. To the diethyl 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 $\langle 1.07 \rangle$ —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 $\langle 1.11 \rangle$ —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 exactly 10 mL of chloroform, and use this solution as the sample solution. Separately, dissolve 50 mg of Tocopherol RS in chloroform to make exactly 100 mL. Pipet 1 mL of this solution, add chloroform to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thinlayer Chromatography $\langle 2.03 \rangle$. Spot 10 μ L each of the sample solution and 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 α - α' -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 sample 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 <2.41> Not more than 2.0% (1 g, in vacuum, phosphorus (V) oxide, 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 sample solution and standard solution. Pipet exactly 20 μ L each of the sample solution and standard solution, and perform the test as directed under Liquid

Chromatography $\langle 2.01 \rangle$ according to the following operating conditions. Determine the peak heights, $H_{\rm T}$ and $H_{\rm S}$, of tocopherol succinate in these solutions, respectively.

Amount (mg) $C_{66}H_{106}CaO_{10} = M_S \times H_T/H_S \times 1.036$

M_S: Amount (mg) of Tocopherol Succinate RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 284 nm).

Column: A stainless steel column about 4 mm in inside diameter and 15 to 30 cm in length, packed with octadecylsilanized silica gel (5 to $10 \,\mu$ L in particle diameter).

Column temperature: Room temperature.

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.

Selection of column: Dissolve 0.05 g each of tocopherol succinate and tocopherol in 50 mL of a mixture of ethanol (99.5) and diluted acetic acid (100) (1 in 5) (9:1). Proceed with 20 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of tocopherol succinate and tocopherol in this order with the resolution between these peaks being not less than 2.0.

System repeatability: Repeat the test 5 times with $20 \,\mu L$ of the standard solution under the above operating conditions: the relative standard deviation of the peak height of tocopherol succinate is not more than 0.8%.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Tocopherol Nicotinate

Vitamin E Nicotinate dl- α -Tocopherol Nicotinate



C₃₅H₅₃NO₃: 535.80 2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yl nicotinate [*51898-34-1*]

To copherol Nicotinate contains not less than 96.0% of $C_{35}H_{53}NO_3$.

Description Tocopherol Nicotinate occurs as a yellow to orange-yellow liquid or solid.

It is freely soluble in ethanol (99.5), and practically insoluble in water.

A solution of Tocopherol Nicotinate in ethanol (99.5) (1 in 10) shows no optical rotation.

It is affected by light.

Identification (1) Determine the absorption spectrum of a solution of Tocopherol Nicotinate in ethanol (99.5) (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Tocopherol Nicotinate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectrum of Tocopherol Nicotinate, if necessary melt by warming, as directed in the liquid film method under the Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Tocopherol Nicotinate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals $\langle 1.07 \rangle$ —Proceed with 1.0 g of Tocopherol Nicotinate 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 $\langle 1.11 \rangle$ —Prepare the test solution with 1.0 g of Tocopherol Nicotinate according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.05 g of Tocopherol Nicotinate in 50 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 7 mL of the sample solution, add ethanol (99.5) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly $10\,\mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> 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 tocopherol nicotinate from the sample solution is not larger than the peak area of tocopherol nicotinate from the standard solution, and the area of a peak which has a retention time 0.8 to 0.9 times that of tocopherol nicotinate from the sample solution is not larger than 4/7 times the peak area of tocopherol nicotinate from the standard solution.

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: A mixture of methanol and water (19:1).

Flow rate: Adjust the flow rate so that the retention time of tocopherol nicotinate is about 20 minutes.

Time span of measurement: About 1.5 times as long as the retention time of tocopherol nicotinate beginning after the solvent peak.

System suitability-

Test for required detection: To exactly 1 mL of the sample solution add ethanol (99.5) 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, add ethanol (99.5) to make exactly 10 mL. Confirm that the peak area of tocopherol nicotinate obtained from $10 \,\mu$ L of this solution is equivalent to 7 to 13% of that from $10 \,\mu$ L of the solution for system suitability test.

System performance: Dissolve 0.05 g of Tocopherol Nicotinate and 0.25 g of tocopherol in 100 mL of ethanol (99.5). When the procedure is run with 10 μ L of this solution under the above operating conditions, tocopherol and tocopherol nicotinate are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of tocopherol nicotinate is not more than 2.0%.

Assay Weigh accurately about 50 mg each of Tocopherol Nicotinate and Tocopherol Nicotinate RS, dissolve each in ethanol (99.5) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.0I \rangle$ according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of tocopherol nicotinate of these solutions.

Amount (mg) of $C_{35}H_{53}NO_3 = M_S \times A_T/A_S$

M_S: Amount (mg) of Tocopherol Nicotinate RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 264 nm).

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

Column temperature: A constant temperature of about $35^{\circ}C$.

Mobile phase: Methanol.

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

System suitability—

System performance: Dissolve 0.05 g of Tocopherol Nicotinate and 0.25 g of tocopherol in 100 mL of ethanol (99.5). When the procedure is run with 5μ L of this solution under the above operating conditions, tocopherol and tocopherol nicotinate 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 μ L of the standard solution under the above operating conditions: the relative standard deviation of the peak areas of tocopherol nicotinate is not more than 0.8%

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Todralazine Hydrochloride Hydrate

Ecarazine Hydrochloride

トドララジン塩酸塩水和物



C₁₁H₁₂N₄O₂.HCl.H₂O: 286.71 Ethyl 2-(phthalazin-1-yl)hydrazinecarboxylate monohydrochloride monohydrate [*3778-76-5*, anhydride]

Todralazine Hydrochloride Hydrate contains not less than 98.5% of todralazine hydrochloride ($C_{11}H_{12}N_4O_2$.HCl: 268.70), calculated on the anhy-

drous basis.

Description Todralazine Hydrochloride Hydrate occurs as white crystals or crystalline powder. It has a slight, characteristic odor, and has a bitter taste.

It is very soluble in formic acid, freely soluble in methanol, soluble in water, sparingly soluble in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of Todralazine Hydrochloride Hydrate (1 in 200) is between 3.0 and 4.0.

Identification (1) To 2 mL of a solution of Todralazine Hydrochloride Hydrate (1 in 200) add 5 mL of silver nitrateammonia TS: the solution becomes turbid, and a black precipitate is formed.

(2) Determine the absorption spectrum of a solution of Todralazine Hydrochloride Hydrate in 0.1 mol/L hydrochloric acid TS (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Todralazine Hydrochloride Hydrate as directed in the potassium chloride disk method under the Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Todralazine Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests $\langle 1.09 \rangle$ (1) for chloride.

Purity (1) Clarity and color of solution—Dissolve 0.30 g of Todralazine Hydrochloride Hydrate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Sulfate $\langle 1.14 \rangle$ —Proceed the test with 2.0 g of Todralazine Hydrochloride Hydrate. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.012%).

(3) Heavy metals $\langle 1.07 \rangle$ —Proceed with 1.0 g of Todralazine 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) Arsenic $\langle 1.11 \rangle$ —Prepare the test solution with 1.0 g of Todralazine Hydrochloride Hydrate according to Method 1, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 50 mg of Todralazine Hydrochloride Hydrate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ 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 the peak of todralazine from the sample solution is not larger than the peak area of todralazine from the standard solution.

Operating conditions—

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

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about

25°C.

Mobile phase: Dissolve 1.10 g of sodium 1-heptane sulfonate in 1000 mL of diluted methanol (2 in 5). Adjust the pH of the solution to between 3.0 and 3.5 with acetic acid (100).

Flow rate: Adjust the flow rate so that the retention time of todralazine is about 8 minutes.

Time span of measurement: About twice as long as the retention time of todralazine beginning after the solvent peak.

System suitability-

Test for required detection: To exactly 5 mL of the standard solution add the mobile phase to make exactly 25 mL. Confirm that the peak area of todralazine obtained from 10 μ L of this solution is equivalent to 15 to 25% of that from 10 μ L of the standard solution.

System performance: Dissolve 5 mg each of Todralazine Hydrochloride Hydrate and potassium biphthalate in 100 mL of the mobile phase. When the procedure is run with 10 μ L of this solution under the above operating conditions, phthalic acid and todralazine are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of todralazine is not more than 2.0%.

Water $\langle 2.48 \rangle$ 6.0 – 7.5% (0.5 g, volumetric titration, direct titration).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Todralazine Hydrochloride, dissolve in 5 mL of formic acid, add 70 mL of acetic anhydride, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

> Each mL of 0.1 mol/L perchloric acid VS = $26.87 \text{ mg of } C_{11}H_{12}N_4O_2.HCl$

Containers and storage Containers—Tight containers.

Tofisopam

トフィソパム



C₂₂H₂₆N₂O₄: 382.45 (5*RS*)-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% of $C_{22}H_{26}N_2O_4$.

Description Tofisopam occurs as a pale yellowish white, crystalline powder.

It is freely soluble in acetic acid (100), soluble in acetone, sparingly soluble in ethanol (95), slightly soluble in diethyl 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 spectrum of a solution of Tofisopam in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tofisopam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 155 – 159°C

Purity (1) Heavy metals $\langle 1.07 \rangle$ —Proceed 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 $\langle 1.11 \rangle$ —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 0.05 g of Tofisopam in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample 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 Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 10 μ L each of the sample 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, 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 standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, in vacuum, silica gel, 60°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). 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.

JP XVI

Tolazamide

トラザミド



C₁₄H₂₁N₃O₃S: 311.40 *N*-(Azepan-1-ylcarbamoyl)-4-methylbenzenesulfonamide [*1156-19-0*]

Tolazamide, when dried, contains not less than 97.5% and not more than 102.0% of $C_{14}H_{21}N_3O_3S$.

Description Tolazamide occurs as a white to pale yellow, crystalline powder. It is odorless.

It is freely soluble in chloroform, soluble in acetone, slightly soluble in ethanol (95) and in n-butylamine, and practically insoluble in water and in diethyl ether.

Melting point: about 168°C (with decomposition).

Identification (1) Dissolve 0.02 g of Tolazamide in 5 mL of water and 1 mL of *n*-butylamine, add 2 to 3 drops of copper (II) sulfate TS, and shake well. Shake well this solution with 5 mL of chloroform, and allow to stand: a green color develops in the chloroform layer.

(2) Determine the absorption spectrum of a solution of Tolazamide in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Tolazamide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Tolazamide, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Tolazamide RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals $\langle 1.07 \rangle$ —Proceed with 1.0 g of Tolazamide 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 $\langle 1.11 \rangle$ —Prepare the test solution with 1.0 g of Tolazamide according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.20 g of Tolazamide in acetone to make exactly 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 200 mL, and use this solution as the standard solution (1). Separately, dissolve 20 mg of *p*-toluenesulfonamide in acetone to make exactly 200 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 10 μ L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, cyclohexane and diluted ammonia solution (28) (10 in 11) (200:100:60:23) to a distance of about 12 cm, and air-dry the plate. Heat the plate at 110°C for 10 minutes, and immediately expose to chlorine for 2 minutes. Expose the plate to cold wind until a very pale blue color develops when 1 drop of potassium iodide-starch TS is placed on a site below the starting line on the plate. Spray evenly potassium iodide-starch TS on the plate: the spot from the sample solution corresponding to the spot from the standard solution (2) is not more intense than the spot from the standard adove spots from the sample solution are not more intense than the spot from the standard solution (2), and the spots other than the principal and above spots from the standard solution (1).

(4) N-Aminohexamethyleneimine—To 0.50 g of Tolazamide add 2.0 mL of acetone, stopper the flask tightly, shake vigorously for 15 minutes. Add 8.0 mL of disodium hydrogenphosphate-citric acid buffer solution, pH 5.4, shake, allow to stand for 15 minutes, and filter. To the filtrate add 1.0 mL of trisodium ferrous pentacyanoamine TS, and shake: the color developing within 30 minutes is not deeper than that of the following control solution.

Control solution: Dissolve 0.125 g of *N*-aminohexamethyleneimine in acetone to make exactly 100 mL. Pipet 1 mL of this solution, and add acetone to make exactly 100 mL. To 2.0 mL of this solution add 8.0 mL of disodium hydrogenphosphate-citric acid buffer solution, pH 5.4, shake, and proceed in the same manner.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.2% (1 g).

Assay Weigh accurately about 30 mg each of Tolazamide and Tolazamide RS, previously dried, dissolve each in exactly 10 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of tolazamide to that of the internal standard, respectively.

Amount (mg) of $C_{14}H_{21}N_3O_3S = M_S \times Q_T/Q_S$

 $M_{\rm S}$: Amount (mg) of Tolazamide RS

Internal standard solution—A solution of tolbutamide in ethanol-free chloroform (3 in 2000).

Operating conditions-

Detector: An ultraviolet absorption photometer (wave-length: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 30 cm in length, packed with silica gel for liquid chromatography (5 μ m in particle diameter).

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

Mobile phase: A mixture of hexane, water-saturated hexane, tetrahydrofuran, ethanol (95) and acetic acid (100) (475:475:20:15:9).

Flow rate: Adjust the flow rate so that the retention time of tolazamide is about 12 minutes.

System suitability-

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the internal standard and tolazamide 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 $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of tolazamide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Tolbutamide

トルブタミド



C₁₂H₁₈N₂O₃S: 270.35 *N*-(Butylcarbamoyl)-4-methylbenzenesulfonamide [64-77-7]

Tolbutamide, when dried, contains not less than 99.0% of $C_{12}H_{18}N_2O_3S$.

Description Tolbutamide occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor. It is tasteless.

It is soluble in ethanol (95), slightly soluble in diethyl 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 reflux 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 <2.60> between 135° C and 139° C.

(2) Render the filtrate obtained in (1) alkaline with about 20 mL of a solution of sodium hydroxide (1 in 5), and heat: an ammonia-like odor is perceptible.

Melting point <2.60> 126 – 132°C

Purity (1) Acidity—Warm 3.0 g of Tolbutamide with 150 mL of water 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 develops.

(2) Chloride $\langle 1.03 \rangle$ —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 using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(3) Sulfate $\langle 1.14 \rangle$ —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 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 0.021%).

(4) Heavy metals $\langle 1.07 \rangle$ —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).

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 3 hours).

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Residue on ignition $\langle 2.44 \rangle$ 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 $\langle 2.50 \rangle$ with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS = $27.04 \text{ mg of } C_{12}H_{18}N_2O_3S$

Containers and storage Containers-Well-closed containers.

Tolbutamide Tablets

トルブタミド錠

Tolbutamide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of tolbutamide ($C_{12}H_{18}N_2O_3S$: 270.35).

Method of preparation Prepare as directed under Tablets, with Tolbutamide.

Identification Shake a quantity of powdered Tolbutamide Tablets, equivalent to 0.5 g of Tolbutamide according to the labeled amount, with 50 mL of chloroform, filter, and evaporate the filtrate to dryness. Proceed with the residue as directed in the Identification under Tolbutamide.

Uniformity of dosage units $\langle 6.02 \rangle$ It meets the requirement of the Mass variation test.

Dissolution $\langle 6.10 \rangle$ When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of phosphate buffer solution, pH 7.4 as the dissolution medium, the dissolution rate in 30 minutes of Tolbutamide Tablets is not less than 80%.

Start the test with 1 tablet of Tolbutamide Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding $0.8 \,\mu\text{m}$. 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 10 μ g of tolbutamide (C₁₂H₁₈N₂O₃S) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Tolbutamide RS, previously dried at 105°C for 3 hours, dissolve in 10 mL of methanol, and add the dissolution medium 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. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the control, and determine the absorbances, $A_{\rm T}$ and $A_{\rm S}$, at 226 nm.

Dissolution rate (%) with respect to the labeled amount of tolbutamide ($C_{12}H_{18}N_2O_3S$)

 $= M_{\rm S} \times A_{\rm T}/A_{\rm S} \times V'/V \times 1/C \times 18$

 $M_{\rm S}$: Amount (mg) of Tolbutamide RS

C: Labeled amount (mg) of tolbutamide $(C_{12}H_{18}N_2O_3S)$ in 1 tablet

Assay Weigh accurately and powder not less than 20 Tol-

butamide Tablets. Weigh accurately a portion of the powder, equivalent to about 0.5 g of tolbutamide ($C_{12}H_{18}N_2O_3S$), dissolve in 50 mL of neutralized ethanol, add 25 mL of water, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

> Each mL of 0.1 mol/L sodium hydroxide VS = $27.04 \text{ mg of } C_{12}H_{18}N_2O_3S$

Containers and storage Containers-Well-closed containers.

Tolnaftate

トルナフタート

C₁₉H₁₇NOS: 307.41 *O*-Naphthalen-2-yl *N*-methyl-*N*-(3-methylphenyl)thiocarbamate [2398-96-1]

Tolnaftate, when dried, contains not less than 98.0% of $C_{19}H_{17}NOS$.

Description Tolnaftate occurs as a white powder. It is odorless.

It is freely soluble in chloroform, sparingly soluble in diethyl ether, slightly soluble in methanol and 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 (II) acetate TS: a black precipitate is formed.

(2) Determine the absorption spectrum of a solution of Tolnaftate in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Tolnaftate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Tolnaftate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Tolnaftate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 111 – 114°C (after drying).

Purity (1) Heavy metals $\langle 1.07 \rangle$ —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. After 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 sample solution. Pipet 2 mL of the sample solution, and add chloroform to make exactly 100 mL. Pipet 5 mL of this solution, add chloroform 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 $\langle 2.03 \rangle$. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with 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 (wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, 65°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ Weigh accurately about 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 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 mL each of the solutions, to each add methanol to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, $A_{\rm T}$ and $A_{\rm S}$, of the sample solution and standard solution at 257 nm as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$.

Amount (mg) of $C_{19}H_{17}NOS = M_S \times A_T/A_S$

 $M_{\rm S}$: Amount (mg) of Tolnaftate RS

Containers and storage Containers—Tight containers.

Tolnaftate Solution

トルナフタート液

Tolnaftate Solution contains not less than 90.0% and not more than 110.0% of the labeled amount of tolnaftate ($C_{19}H_{17}NOS$: 307.41).

Method of preparation Prepare as directed under Liquids and Solutions for Cutaneous Application, with Tolnaftate.

Identification (1) Spot 1 drop of Tolnaftate Solution on filter paper. Spray hydrogen hexachloroplatinate (IV)-potas-

sium iodide TS on the paper: a light yellow color develops in the spot.

(2) To a volume of Tolnaftate Solution, equivalent to 0.02 g of Tolnaftate according to the labeled amount, add chloroform to make 10 mL, and use this solution as the sample solution. Separately, dissolve 0.02 g of Tolnaftate RS in 10 mL of chloroform, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with toluene to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution and that from the standard solution show the same Rf value.

Assay Pipet a volume of Tolnaftate Solution, equivalent to about 20 mg of tolnaftate ($C_{19}H_{17}NOS$), add exactly 4 mL of the internal standard solution, then add chloroform to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of Tolnaftate RS, previously dried in vacuum at a pressure not exceeding 0.67 kPa at 65°C for 3 hours, and dissolve in chloroform to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 4 mL of the internal standard solution, then add chloroform to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.0I \rangle$ according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of tolnaftate to that of the internal standard, respectively.

> Amount (mg) of tolnaftate (C₁₉H₁₇NOS) = $M_{\rm S} \times Q_{\rm T}/Q_{\rm S} \times 1/20$

 $M_{\rm S}$: Amount (mg) of Tolnaftate RS

Internal standard solution—A solution of diphenyl phthalate in chloroform (3 in 200).

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-length: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and 15 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to $10 \,\mu$ m in particle diameter).

Column temperature: A constant temperature of about $25^{\circ}C$.

Mobile phase: A mixture of methanol and water (7:3).

Flow rate: Adjust the flow rate so that the retention time of tolnaftate is about 14 minutes.

Selection of column: Proceed with $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of the internal standard and tolnaftate in this order with the resolution between these peaks being not less than 5.

Containers and storage Containers—Tight containers.

Tolperisone Hydrochloride





C₁₆H₂₃NO.HCl: 281.82 (*2RS*)-2-Methyl-1-(4-methylphenyl)-3-piperidin-1ylpropan-1-one monohydrochloride [*3644-61-9*]

Tolperisone Hydrochloride, when dried, contains not less than 98.5% of $C_{16}H_{23}NO.HCl$.

Description Tolperisone Hydrochloride occurs as a white, crystalline powder. It has a slight, characteristic odor.

It is very soluble in acetic acid (100), freely soluble in water and in ethanol (95), soluble in acetic anhydride, slightly soluble in acetone, and practically insoluble in diethyl ether.

The pH of a solution of Tolperisone Hydrochloride (1 in 20) is between 4.5 and 5.5.

It is hygroscopic.

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 develops.

(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 ammonia TS, and filter. Acidify 5 mL of the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests $\langle 1.09 \rangle$ for chloride.

Absorbance $\langle 2.24 \rangle = E_{1 \text{ cm}}^{1\%}$ (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 $\langle 1.14 \rangle$ —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 <1.07>—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 sample solution. Separately, dissolve 20 mg of piperidine hydrochloride in water to make exactly 1000 mL, and use this solution as the standard solution. Transfer 5.0 mL each of the sample solution and standard solution to different separators, add 0.1 mL each of a solution of copper (II) sulfate pentahydrate (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 dehydrate with anhydrous sodium sulfate. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: the absorbance of the sample solution at 438 nm is not more than that of the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, in vacuum, silica gel, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Tolperisone Hydrochloride, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

> Each mL of 0.1 mol/L perchloric acid VS = $28.18 \text{ mg } C_{16}H_{23}NO.HCl$

Containers and storage Containers-Well-closed containers.

Tosufloxacin Tosilate Hydrate

トスフロキサシントシル酸塩水和物



 $C_{19}H_{15}F_3N_4O_3.C_7H_8O_3S.H_2O: 594.56$

7-[(3*RS*)-3-Aminopyrrolidin-1-yl]-1-(2,4-difluorophenyl)-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid mono-4-toluenesulfonate monohydrate [*115964-29-9*, anhydride]

Tosufloxacin Tosilate Hydrate contains not less than 98.5% and not more than 101.0% of tosufloxacin tosilate ($C_{19}H_{15}F_3N_4O_3.C_7H_8O_3S$: 576.54), calculated on the anhydrous basis.

Description Tosufloxacin Tosilate Hydrate occurs as a white to pale yellowish white, crystalline powder.

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

A solution of Tosufloxacin Tosilate Hydrate in methanol (1 in 100) shows no optical rotation.

Melting point: about 254°C (with decomposition).

Identification (1) Tosufloxacin Tosilate Hydrate shows a light bluish-white fluorescence under ultraviolet light (main wavelength 254 nm).

(2) Proceed 10 mg of Tosufloxacin Tosilate Hydrate as directed under Oxygen Flask Combustion Method $\langle 1.06 \rangle$, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the solution

responds to the Qualitative Tests <1.09> (2) for fluoride.

(3) Determine the absorption spectrum of a solution of Tosufloxacin Tosilate Hydrate in a mixture of methanol and sodium hydroxide TS (49:1) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Tosufloxacin Tosilate Hydrate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Tosufloxacin Tosilate Hydrate as directed in the paste method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Tosufloxacin Tosilate Hydrate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Chloride $\langle 1.03 \rangle$ —Dissolve 1.0 g of Tosufloxacin Tosilate Hydrate in 40 mL of *N*,*N*-dimethylformamide, and add 6 mL of dilute nitric acid and *N*,*N*-dimethylformamide to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS, 6 mL of dilute nitric acid and *N*,*N*-dimethylformamide to make 50 mL (not more than 0.007%).

(2) Heavy metals $\langle 1.07 \rangle$ —Proceed with 1.0 g of Tosufloxacin Tosilate Hydrate 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 $\langle 1.11 \rangle$ —Prepare the test solution with 1.0 g of Tosufloxacin Tosilate Hydrate according to Method 4, and perform the test under the condition of the ignition temperature being between 750 and 850°C, and add 10 mL of diluted hydrochloric acid to residue (not more than 2 ppm).

(4) Related substances—Dissolve 10 mg of Tosufloxacin Tosilate Hydrate in 12 mL of mobile phase B, add water to make 25 mL, and use this solution as the sample solution. Pipet 5 mL of the sample solution, and add mobile phase A to make exactly 100 mL. Pipet 2 mL of this solution, add mobile phase A to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by automatic integration method: the area of each peak other than tosylic acid and tosufloxacin obtained from the sample solution is not larger than 3/4 times the peak area of tosufloxacin from the standard solution, and the total area of the peaks other than tosylic acid and tosufloxacin from the sample solution is not larger than 2.5 times the peak area of tosufloxacin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 272 nm).

Column: A stainless steel column 3.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

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

Mobile phase A: To 300 to 500 mL of water add slowly 100 mL of methanesulfonic acid under ice-cooling, add slowly 100 mL of triethylamine under ice-cooling too, and add water to make 1000 mL. To 10 mL of this solution add 143 mL of water, 40 mL of acetonitrile and 7 mL of 1 mol/L dipotassium hydrogen phosphate TS for buffer solution.

Mobile phase B: To 300 to 500 mL of water add slowly 100 mL of methanesulfonic acid under ice-cooling, add slowly 100 mL of triethylamine under ice-cooling too, and add water to make 1000 mL. To 10 mL of this solution add 100 mL of acetonitrile, 83 mL of water and 7 mL of 1 mol/L dipotassium hydrogen phosphate TS for buffer solution.

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

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 - 1	100	0
1 - 16	$100 \rightarrow 0$	$0 \rightarrow 100$
16 - 35	0	100

Flow rate: 0.5 mL per minute.

Time span of measurement: About 5 times as long as the retention time of tosufloxacin.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add mobile phase A to make exactly 20 mL. Confirm that the peak area of tosufloxacin obtained from 20 μ L of this solution is equivalent to 18 to 32% of the peak area of tosufloxacin from 20 μ L of the standard solution.

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of tosufloxacin are not less than 10,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tosufloxacin is not more than 2.0%.

(5) Residual solvent—Being specified separately.

Water $\langle 2.48 \rangle$ 2.5 – 3.5% (30 mg, coulometric titration).

Assay Weigh accurately about 30 mg each of Tosufloxacin Tosilate Hydrate and Tosufloxacin Tosilate RS (separately determine the water $\langle 2.48 \rangle$ in the same manner as Tosufloxacin Tosilate Hydrate), and dissolve each in methanol to make exactly 100 mL. Pipet 20 mL each of these solutions, to each add exactly 4 mL of the internal standard solution and methanol to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of tosufloxacin to that of the internal standard.

Amount (mg) of tosufloxacin tosilate $(C_{19}H_{15}F_3N_4O_3.C_7H_8O_3S)$ $= M_S \times Q_T/Q_S$

 $M_{\rm S}$: Amount (mg) of Tosufloxacin Tosilate RS, calculated on the anhydrous basis

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (1 in 800). *Operating conditions*— Detector: An ultraviolet absorption photometer (wavelength: 270 nm).

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

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

Mobile phase: To a mixture of 0.02 mol/L phosphate buffer solution, pH 3.5, and a solution of dibutylamine in methanol (1 in 2500) (3:1) add diluted phosphoric acid (1 in 10) to adjust the pH to 3.5.

Flow rate: Adjust the flow rate so that the retention time of tosufloxacin is about 20 minutes.

System suitability-

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the internal standard and tosufloxacin 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 $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of tosufloxacin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Tosufloxacin Tosilate Tablets

トスフロキサシントシル酸塩錠

Tosufloxacin Tosilate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of tosuflozacin tosilate hydrate ($C_{19}H_{15}F_3N_4O_3.C_7H_8O_3S.H_2O$: 594.56).

Method of preparation Prepare as directed under Tablets, with Tosufloxacin Tosilate Hydrate.

Identification To a quantity of powdered Tosufloxacin Tosilate Tablets, equivalent to 75 mg of Tosufloxacin Tosilate Hydrate according to the labeled amount, add 200 mL of a mixture of methanol and sodium hydroxide TS (49:1), shake well, and centrifuge. To 2 mL of the supernatant liquid add 100 mL of a mixture of methanol and sodium hydroxide TS (49:1). Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: it exhibits maxima between 260 nm and 264 nm, between 341 nm and 345 nm, and between 356 nm and 360 nm.

Uniformity of dosage units $\langle 6.02 \rangle$ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Tosufloxacin Tosilate Tablets add V/10 mL of water and shake until the tablet is disintegrated. Add methanol to make exactly V mL so that each mL contains about 1.5 mg of tosufloxacin tosilate hydrate ($C_{19}H_{15}F_3N_4O_3$. $C_7H_8O_3S.H_2O$). Shake this solution for 10 minutes, and centrifuge. Pipet 4 mL of the supernatant liquid, add exactly 4 mL of the internal standard solution and methanol to make 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

1518 Tranexamic Acid / Official Monographs

Amount (mg) of tosufloxacin tosilate hydrate $(C_{19}H_{15}F_3N_4O_3.C_7H_8O_3S.H_2O)$ $= M_S \times Q_T/Q_S \times V/20 \times 1.031$

 $M_{\rm S}$: Amount (mg) of Tosufloxacin Tosilate RS, calculated on the anhydrous basis

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (1 in 800).

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 90 minutes of Tosufloxacin Tosilate Tablets is not less than 65%.

Start the test with 1 tablet of Tosufloxacin Tosilate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding $0.5 \,\mu\text{m}$. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, to make exactly V' mL so that each mL contains about $17 \mu g$ of tosufloxacin tosilate hydrate (C₁₉H₁₅F₃N₄O₃.C₇H₈O₃S.H₂O) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 21 mg of Tosufloxacin Tosilate RS (separately determine the water $\langle 2.48 \rangle$ in the same manner as Tosufloxacin Tosilate Hydrate), and dissolve in N,N-dimethylformamide to make exactly 25 mL. Pipet 2 mL of this solution, add 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, as the blank, and determine the absorbances, $A_{\rm T}$ and $A_{\rm S}$, at 346 nm.

Dissolution rate (%) with respect to the labeled amount of tosufloxacin tosilate hydrate (C₁₉H₁₅F₃N₄O₃.C₇H₈O₃S.H₂O) = $M_{\rm S} \times A_{\rm T}/A_{\rm S} \times V'/V \times 1/C \times 72 \times 1.031$

- $M_{\rm S}$: Amount (mg) of Tosufloxacin Tosilate RS, calculated on the anhydrous basis
- C: Labeled amount (mg) of tosufloxacin tosilate hydrate (C₁₉H₁₅F₃N₄O₃.C₇H₈O₃S.H₂O) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Tosufloxacin Tosilate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.15 g of tosufloxacin tosilate hydrate (C19H15F3N4O3.C7H8O3S.H2O), add 10 mL of water and methanol to make exactly 100 mL, shake for 10 minutes, and centrifuge. Pipet 4 mL of the supernatant liquid, add exactly 4 mL of the internal standard solution and methanol to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Tosufloxacin Tosilate RS (separately determine the water <2.48> in the same manner as Tosufloxacin Tosilate Hydrate), add 2 mL of water, and dissolve in methanol to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 4 mL of the internal standard solution and methanol to make 100 mL, and use this solution as the standard solution. Perform the test with $10 \,\mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of tosufloxacin to that of the internal standard.

Amount (mg) of tosufloxacin tosilate hydrate $(C_{19}H_{15}F_{3}N_{4}O_{3}.C_{7}H_{8}O_{3}S.H_{2}O)$ $= M_{S} \times Q_{T}/Q_{S} \times 5 \times 1.031$

 $M_{\rm S}$: Amount (mg) of Tosufloxacin Tosilate RS, calculated on the anhydrous basis

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (1 in 800).

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Tosufloxacin Tosilate Hydrate.

System suitability-

Proceed as directed in the system suitability in the Assay under Tosufloxacin Tosilate Hydrate.

Containers and storage Containers-Well-closed containers.

Tranexamic Acid

トラネキサム酸



C₈H₁₅NO₂: 157.21

trans-4-(Aminomethyl)cyclohexanecarboxylic acid [1197-18-8]

Tranexamic Acid, when dried, contains not less than 98.0% and not more than 101.0% of $C_8H_{15}NO_2$.

Description Tranexamic Acid occurs as white crystals or crystalline powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

Identification Determine the infrared absorption spectrum of Tranexamic Acid as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Tranexamic Acid RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH $\langle 2.54 \rangle$ The pH of a solution prepared by dissolving 1.0 g of Tranexamic Acid in 20 mL of water 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 $\langle 1.03 \rangle$ —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 sample stock solution. To 12 mL of the sample stock solution add 2 mL of hydrochloric acid-ammonium acetate buffer solution, pH 3.5, mix, add 1.2 mL of thioacetamide TS, mix immediately, and use this solution as the sample solution. Sepa-

rately, proceed in the same manner as above with a mixture of 1 mL of Standard Lead Solution, 2 mL of the sample 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 sample 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 sample solution and the standard solution 2 minutes after they are prepared: the color of the sample solution is not more intense than that of the standard solution (not more than 10 ppm).

(4) Arsenic $\langle 1.11 \rangle$ —Prepare the test solution by dissolving 1.0 g of Tranexamic Acid in 10 mL of water, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.20 g of Tranexamic Acid in 20 mL of water, and use this solution as the sample solution. Pipet 5 mL of the sample 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 $20 \,\mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> 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 tranexamic acid obtained from sample solution, is not larger than 2/5 times 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, is not larger than 1/5 times 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 larger than 1/5 times the peak area of tranexamic acid from the standard solution. For this comparison, use the area 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 larger 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.

Time span of measurement: About 3 times as long as the retention time of tranexamic acid beginning after the solvent peak.

System suitability-

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 μ L of this solution is equivalent to 14 to 26% of that from 20 μ L of the standard solution.

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

System repeatability: When the test is repeated 6 times with $20 \,\mu\text{L}$ 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%.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Tranexamic Acid and Tranexamic Acid RS, previously dried, dissolve in water to make exactly 25 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of tranexamic acid.

Amount (mg) of $C_8H_{15}NO_2 = M_S \times A_T/A_S$

M_S: Amount (mg) of Tranexamic Acid RS

Operating conditions-

Detector: An ultraviolet absorption photometer (wave-length: 220 nm).

Column: A stainless steel column 6.0 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

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

Mobile phase: Dissolve 11.0 g of anhydrous 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 μ L of this solution under the above operating conditions, 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 $20 \,\mu\text{L}$ of the standard solution under 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.

Tranexamic Acid Capsules

トラネキサム酸カプセル

Tranexamic Acid Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of tranexamic acid ($C_8H_{15}NO_2$: 157.21).

Method of preparation Prepare as directed under Capsules, with Tranexamic Acid.

Identification Take an amount of powdered contents of Tranexamic Acid Capsules, equivalent to 0.5 g of Tranexamic Acid according to the labeled amount, add 50 mL of water, shake well, and filter. To 5 mL of the filtrate add 1 mL of ninhydrin TS, and heat for 3 minutes: a dark purple color develops.

Uniformity of dosage units <6.02> It meets the requirement

of the Mass variation test.

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Tranexamic Acid Capsules is not less than 80%.

Start the test with 1 tablet of Tranexamic Acid Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding $0.45 \,\mu\text{m}$. 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 0.28 mg of tranexamic acid (C₈H₁₅NO₂) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Tranexamic Acid RS, previously dried at 105°C for 2 hours, dissolve in water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas of tranexamic acid, $A_{\rm T}$ and $A_{\rm S}$.

Dissolution rate (%) with respect to the labeled amount of tranexamic acid ($C_8H_{15}NO_2$)

 $= M_{\rm S} \times A_{\rm T}/A_{\rm S} \times V'/V \times 1/C \times 900$

 $M_{\rm S}$: Amount (mg) of Tranexamic Acid RS

C: Labeled amount (mg) of tranexamic acid ($C_8H_{15}NO_2$) in 1 capsule

Operating conditions—

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

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

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

Mobile phase: Dissolve 11.0 g of anhydrous sodium dihydrogen phosphate in 500 mL of water, and add 10 mL of triethylamine and 1.4 g of sodium lauryl sulfate. Adjust the pH to 2.5 with phosphoric acid, 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 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of tranexamic acid are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tranexamic acid is not more than 2.0%.

Assay Weigh accurately the mass of the contents of not less than 20 Tranexamic Acid Capsules, and powder. Weigh accurately an amount of the powder, equivalent to about 0.1 g of tranexamic acid ($C_8H_{15}NO_2$), add 30 mL of water, shake well, and add water to make exactly 50 mL. Centrifuge, filter the supernatant liquid through a membrane filter with pore size of not more than 0.45 μ m, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of Tranexamic Acid RS, previously dried at 105 °C for 2 hours, dissolve in water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 30 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of tranexamic acid.

Amount (mg) of tranexamic acid (C₈H₁₅NO₂) = $M_{\rm S} \times A_{\rm T}/A_{\rm S} \times 2$

M_S: Amount (mg) of Tranexamic Acid RS

Operating conditions—

Detector, column, and mobile phase: Proceed as directed in the operating conditions in the Assay under Tranexamic Acid.

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

Flow rate: Adjust the flow rate so that the retention time of tranexamic acid is about 16 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 30 μ L of this solution under the above operating conditions, tranexamic acid and 4-(aminomethyl)benzoic acid 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 $30 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tranexamic acid is not more than 1.0%.

Containers and storage Containers—Tight containers.

Tranexamic Acid Injection

トラネキサム酸注射液

Tranexamic Acid Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of tranexamic acid ($C_8H_{15}NO_2$: 157.21).

Method of preparation Prepare as directed under Injections, with Tranexamic Acid.

Description Tranexamic Acid Injection is a clear and color-less liquid.

Identification To a volume of Tranexamic Acid Injection, equivalent to 50 mg of Tranexamic Acid according to the labeled amount, add water to make 5 mL, add 1 mL of ninhydrin TS, and heat: a dark purple color develops.

pH <2.54> 7.0 - 8.0

Bacterial endotoxins <4.01> Not more than 0.12 EU/mg.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter $\langle 6.06 \rangle$ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Take accurately a volume of Tranexamic Acid Injection, equivalent to about 0.1 g of tranexamic acid (C₈H₁₅NO₂), add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Tranexamic Acid RS, previously dried at 105 °C for 2 hours, dissolve in water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 30 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of tranexamic acid.

Amount (mg) of tranexamic acid (C₈H₁₅NO₂) = $M_{\rm S} \times A_{\rm T}/A_{\rm S} \times 2$

M_S: Amount (mg) of Tranexamic Acid RS

Operating conditions—

Detector, column, and mobile phase: Proceed as directed in the operating conditions in the Assay under Tranexamic Acid.

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

Flow rate: Adjust the flow rate so that the retention time of tranexamic acid is about 16 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 $30 \,\mu$ L of this solution under the above operating conditions, tranexamic acid and 4-(aminomethyl)benzoic acid 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 $30 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tranexamic acid is not more than 1.0%.

Containers and storage Containers-Hermetic containers.

Tranexamic Acid Tablets

トラネキサム酸錠

Tranexamic Acid Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of tranexamic acid ($C_8H_{15}NO_2$: 157.21).

Method of preparation Prepare as directed under Tablets, with Tranexamic Acid.

Identification To an amount of powdered Tranexamic Acid Tablets, equivalent to 0.5 g of Tranexamic Acid according to the labeled amount, add 50 mL of water, shake well, and filter. To 5 mL of the filtrate add 1 mL of ninhydrin TS, and heat for 3 minutes: a dark purple color develops.

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Dissolution Being specified separately.

Assay Weigh accurately the mass of not less than 20 Tranexamic Acid Tablets, and powder. Weigh accurately a quantity of the powder, equivalent to about 5 g of tranexamic acid (C₈H₁₅NO₂), add 150 mL of water, disintegrate the tablets completely with the aid of ultrasonic waves, and add water to make exactly 200 mL. Centrifuge, pipet 4 mL of the supernatant liquid, and add water to make exactly 50 mL. Filter through a membrane filter with pore size of not more than 0.45 μ m, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of Tranexamic Acid RS, previously dried at 105°C for 2 hours, dissolve in water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly $30 \,\mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of tranexamic acid.

> Amount (mg) of tranexamic acid (C₈H₁₅NO₂) = $M_{\rm S} \times A_{\rm T}/A_{\rm S} \times 100$

 $M_{\rm S}$: Amount (mg) of Tranexamic Acid RS

Operating conditions—

Detector, column, and mobile phase: Proceed as directed in the operating conditions in the Assay under Tranexamic Acid.

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

Flow rate: Adjust the flow rate so that the retention time of tranexamic acid is about 16 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 30 μ L of this solution under the above operating conditions, tranexamic acid and 4-(aminomethyl)benzoic acid 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 $30 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tranexamic acid is not more than 1.0%.

Containers and storage Containers—Tight containers.

Trapidil

トラピジル



C₁₀H₁₅N₅: 205.26 7-Diethylamino-5-methyl[1,2,4]triazolo[1,5-*a*]pyrimidine [*15421-84-8*]

Trapidil, when dried, contains not less than 98.5% of $C_{10}H_{15}N_5$.

Description Trapidil occurs as a white to pale yellowish

white, crystalline powder.

It is very soluble in water and in methanol, freely soluble in ethanol (95), in acetic anhydride and in acetic acid (100), and sparingly soluble in diethyl ether.

The pH of a solution of Trapidil (1 in 100) 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 develops.

(2) Determine the absorption spectrum of a solution of Trapidil (1 in 125,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Absorbance $\langle 2.24 \rangle = E_{1 \text{ cm}}^{1\%}$ (307 nm): 860 – 892 (after drying, 20 mg, water, 2500 mL).

Melting point <2.60> 101 – 105°C

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 $\langle 1.03 \rangle$ —Perform the test with 0.5 g of Trapidil. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

(3) Ammonium—Place 0.05 g of Trapidil in a glassstoppered conical 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 $\langle 1.07 \rangle$ —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 $\langle 1.11 \rangle$ —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 sample solution. Pipet 1 mL of the sample 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 these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 20 μ L each of the sample solution and 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 airdry the plate. Allow the plate to stand in iodine vapor for 60 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, in vacuum, silica gel, 60°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric

titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = $20.53 \text{ mg of } C_{10}H_{15}N_5$

Containers and storage Containers-Tight containers.

Trehalose Hydrate

トレハロース水和物



C12H22O11.2H2O: 378.33

 α -D-Glucopyranosyl α -D-glucopyranoside dihydrate [6138-23-4]

Trehalose Hydrate contains not less than 98.0% and not more than 101.0% of trehalose (C₁₂H₂₂O₁₁: 342.30), calculated on the anhydrous basis.

Description Trehalose Hydrate occurs as white crystals or a white crystalline powder.

It is freely soluble in water, and slightly soluble in methanol and in ethanol (99.5).

Identification (1) To 1 mL of a solution of Trehalose Hydrate (2 in 5) add 5 - 6 drops of a solution of 1-naphthol in ethanol (95) (1 in 20), shake thoroughly, and add gently 2 mL of sulfuric acid: a purple color appears at the zone of contact.

(2) Mix 2 mL of a solution of Trehalose Hydrate (1 in 25) with 1 mL of dilute hydrochloric acid, and allow standing for 20 minutes at room temperature. Then add 4 mL of sodium hydroxide TS and 2 mL of a solution of glycine (1 in 25), and heat in a water bath for 10 minutes: no brown color appears.

(3) Determine the infrared absorption spectrum of Trehalose Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Trehalose RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_D^{20}$: +197 - +201° (10 g, calculated on the anhydrous basis, water, 100 mL, 100 mm).

pH $\langle 2.54 \rangle$ The pH of a solution of 1 g of Trehalose Hydrate in 10 mL of water is between 4.5 and 6.5.

Purity (1) Chloride $\langle 1.03 \rangle$ —Perform the test with 2.0 g of Trehalose Hydrate. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

(2) Sulfate $\langle 1.14 \rangle$ —Perform the test with 2.0 g of Trehalose Hydrate. Prepare the control solution with 1.0 mL of

JP XVI

0.005 mol/L sulfuric acid VS (not more than 0.024%).

(3) Heavy metals $\langle 1.07 \rangle$ —Proceed with 5.0 g of Trehalose 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).

(4) Related substances—Dissolve 0.5 g of Trehalose Hydrate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly $20 \,\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the total area of the peaks which are eluted before the peak of trehalose and the total area of the peaks which are eluted after the peak of trehalose obtained from the sample solution are both not larger than 1/2 times the peak area of trehalose from the standard solution.

Operating conditions—

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

Time span of measurement: About 2 times as long as the retention time of trehalose.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add water to make exactly 10 mL. Confirm that the peak area of trehalose obtained with 20 μ L of this solution is equivalent to 7 to 13% of that with 20 μ L of the standard solution.

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

System repeatability: To exactly 5 mL of the standard solution add water to make exactly 10 mL. When the test is repeated 6 times with 20 μ L of this solution under the above operating conditions, the relative standard deviation of the peak area of trehalose is not more than 1.0%.

(5) Dextrin, soluble starch, and sulfite—Dissolve 1.0 g of Trehalose Hydrate in 10 mL of water and add 1 drop of iodine TS: a yellow color appears, which is changed to blue on addition of 1 drop of starch TS.

(6) Nitrogen—Perform the test with accurately weighed Trehalose Hydrate of about 5 g as directed under Nitrogen Determination $\langle 1.08 \rangle$, using 30 mL of sulfuric acid for the degradation and adding 45 mL of sodium hydroxide solution (2 in 5): the amount of nitrogen (N: 14.01) is not more than 0.005%.

Water $\langle 2.48 \rangle$ not less than 9.0% and not more than 11.0% (0.1 g, volumetric titration, direct titration).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (2 g).

Assay Weigh accurately about 0.2 g each of Trehalose Hydrate and Trehalose RS (separately determine the water $\langle 2.48 \rangle$ in the same manner as Trehalose Hydrate), dissolve each in 6 mL of water, add exactly 2 mL each of the internal standard solution, add water to make them 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of trehalose to that of the internal standard.

Amount (mg) of trehalose ($C_{12}H_{22}O_{11}$) = $M_S \times Q_T/Q_S$

 $M_{\rm S}$: Amount (mg) of Trehalose RS, calculated on the anhydrous basis

Internal standard solution—A solution of glycerin (1 in 10). *Operating conditions*—

Detector: A differential refractometer.

Column: A stainless steel column 8 mm in inside diameter and 30 cm in length, packed with strongly acidic ionexchange resin for liquid chromatography consist of styrenedivinylbenzene copolymer carrying sulfonic acid groups (6 μ m in particle diameter).

Column temperature: A constant temperature of about 80°C.

Mobile phase: Water.

Flow rate: Adjust the flow rate so that the retention time of trehalose is about 15 minutes.

System suitability—

System performance: Dissolve 0.1 g each of maltotriose and glucose in 10 mL of the standard solution, add 1 mL of the internal standard solution, and add water to make 20 mL. When the procedure is run with $20 \,\mu$ L of this solution under the above operating conditions, maltotriose, trehalose, glucose and the internal standard are eluted in this order, and the resolution between the peaks of maltotriose and trehalose is not less than 1.5, the resolution between the peaks of trehalose and glucose is not less than 4, and the resolution between the peaks of glucose and the internal standard is not less than 3.

System repeatability: When the test is repeated 6 times with $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of trehalose to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Trepibutone

トレピブトン



C₁₆H₂₂O₆: 310.34 4-Oxo-4-(2,4,5-triethoxyphenyl)butanoic acid [*41826-92-0*]

Trepibutone, when dried, contains not less than 98.5% of $C_{16}H_{22}O_6$.

Description Trepibutone occurs as white to yellowish white crystals or crystalline powder. It is odorless, and is tasteless or has a slight, characteristic aftertaste.

It is soluble in acetone, sparingly soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Identification (1) Determine the absorption spectrum of a solution of Trepibutone in diluted dilute sodium hydroxide TS (1 in 10) (1 in 100,000) as directed under Ultraviolet-

visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the ¹H spectrum of a solution of Trepibutone in deuterated chloroform for the nuclear magnetic resonance spectroscopy (1 in 10), using tetramethylsilane for the nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy $\langle 2.21 \rangle$: it exhibits a sharp multiple signal A at around δ 1.5 ppm, a triplet signal B at around δ 2.7 ppm, a triplet signal C at around δ 3.3 ppm, a multiple signal D at around δ 4.2 ppm, a sharp single signal E at around δ 6.4 ppm, a sharp single signal F at around δ 7.4 ppm, and a single signal G at around δ 10.5 ppm. The ratio of integrated intensity of each signal, A:B:C:D:E:F:G, is about 9:2:2:6:1:1:1.

Melting point <2.60> 146 – 150°C

Purity (1) Chloride $\langle 1.03 \rangle$ —Dissolve 0.5 g of Trepibutone 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 as follows: to 0.30 mL of 0.01 mol/L hydrochloric acid VS add 30 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%).

(2) Heavy metals $\langle 1.07 \rangle$ —Proceed with 1.0 g of Trepibutone 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 Trepibutone in 10 mL of acetone, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add acetone to make exactly 100 mL. To exactly 10 mL of this solution add acetone 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 $\langle 2.03 \rangle$. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of isopropylether, acetone, water and formic acid (100:30:3:3) 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 sample solution are not more intense than the spot from the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Trepibutone, previously dried, dissolve in 50 mL of ethanol (95), add 50 mL of water, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = 31.03 mg of $C_{16}H_{22}O_6$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Triamcinolone

トリアムシノロン



 $C_{21}H_{27}FO_6$: 394.43 9-Fluoro-11 β ,16 α ,17,21-tetrahydroxypregna-1,4-diene-3,20-dione [*124-94-7*]

Triamcinolone, when dried, contains not less than 97.0% and not more than 103.0% of $C_{21}H_{27}FO_6$.

Description Triamcinolone occurs as a white, crystalline powder. It is odorless.

It is freely soluble in N,N-dimethylformamide, slightly soluble in methanol, in ethanol (95) and in acetone, and practically insoluble in water, in 2-propanol and in diethyl 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-*tert*-butylcresol TS and 5 mL of sodium hydroxide TS, and heat on a water bath for 30 minutes under a reflux condenser: a red-purple color develops.

(2) Add 5 mL of water and 1 mL of Fehling's TS to 0.01 g of Triamcinolone, and heat: a red precipitate is produced.

(3) Proceed with 0.01 g of Triamcinolone as directed under Oxygen Flask Combustion Method $\langle 1.06 \rangle$, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the solution responds to the Qualitative Tests $\langle 1.09 \rangle$ for fluoride.

(4) Determine the infrared absorption spectrum of Triamcinolone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Triamcinolone RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve 0.1 g each of Triamcinolone and Triamcinolone RS in 7 mL of a mixture of 2-propanol and water (2:1), respectively, by warming. Allow the solutions to cool in ice to effect crystals, filter, then wash the formed crystals with two 10-mL portions of water, and repeat the test on the dried crystals.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_D^{20}$: +65 – +71° (after drying, 0.1 g, *N*,*N*-dimethylformamide, 10 mL, 100 mm).

Purity Heavy metals $\langle 1.07 \rangle$ —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 $\langle 2.41 \rangle$ Not more than 2.0% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 exactly 5 mL each of the internal standard solution, add a solution of L-ascorbic acid in methanol (1 in 1000) to make 20 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak height of triamcinolone to that of the internal standard, respectively.

Amount (mg) of $C_{21}H_{27}FO_6 = M_S \times Q_T/Q_S$

$M_{\rm S}$: Amount (mg) of Triamcinolone RS

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 (wave-length: 254 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel (5 μ m in particle diameter).

Column temperature: A constant temperature of about $25^{\circ}C$.

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 μ L 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 $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height 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

トリアムシノロンアセトニド



 $C_{24}H_{31}FO_6$: 434.50 9-Fluoro-11 β ,21-dihydroxy-16 α ,17-(1-methylethylidenedioxy)pregna-1,4-diene-3,20-dione [76-25-5]

Triamcinolone Acetonide, when dried, contains not less than 97.0% and not more than 103.0% of $C_{24}H_{31}FO_6$.

Description Triamcinolone Acetonide occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in ethanol (99.5), in acetone, and in 1,4-dioxane, slightly soluble in methanol and in ethanol (95), and practically insoluble in water and in diethyl 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-*tert*-butylcresol TS and 5 mL of sodium hydroxide TS, and heat on a water bath under a reflux condenser for 20 minutes: a green color develops.

(2) Add 5 mL of water and 1 mL of Fehling's TS to 0.01 g of Triamcinolone Acetonide, and heat: a red precipitate is produced.

(3) Proceed with 0.01 g of Triamcinolone Acetonide as directed under Oxygen Flask Combustion Method $\langle 1.06 \rangle$, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the solution responds to the Qualitative Tests $\langle 1.09 \rangle$ for fluoride.

(4) Determine the absorption spectrum of a solution of Triamcinolone Acetonide in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Triamcinolone Acetonide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(5) Determine the infrared absorption spectrum of Triamcinolone Acetonide, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Triamcinolone Acetonide RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve 0.1 g each of Triamcinolone Acetonide and Triamcinolone Acetonide RS in 20 mL of ethanol (95), respectively, then evaporate the ethanol to dryness, and repeat the test on the dried residue.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_{D}^{20}$: +100 - +107° (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

Purity (1) Heavy metals $\langle 1.07 \rangle$ —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 sample solution. Pipet 1 mL of the sample solution, add acetone 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 <2.03>. Spot 20 μ L each of the sample solution and 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 (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 sample solution are not more intense than the spot from the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 2.0% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 20 μ L each of these solutions as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak height of triamcinolone acetonide to that of the internal standard, respectively.

Amount (mg) of $C_{24}H_{31}FO_6 = M_S \times Q_T/Q_S$

M_S: Amount (mg) of Triamcinolone Acetonide RS

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 4.6 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel (10 μ m in particle diameter).

Column temperature: A constant temperature of about $25^{\circ}C$.

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 μ L 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 $10 \,\mu\text{L}$ of the 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

トリアムテレン



C₁₂H₁₁N₇: 253.26 6-Phenylpteridine-2,4,7-triamine [*396-01-0*]

Triamterene, when dried, contains not less than 98.5% of $C_{12}H_{11}N_7$.

Description Triamterene occurs as a yellow, crystalline powder. It is odorless, and tasteless.

It is sparingly soluble in dimethylsulfoxide, very slightly soluble in acetic acid (100), and practically insoluble in water, in ethanol (95), and in diethyl ether.

It dissolves in nitric acid and in sulfuric acid, but does not dissolve in dilute nitric acid, in dilute sulfuric acid and in dilute hydrochloric acid.

Identification (1) To 0.01 g 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 $\langle 1.09 \rangle$ for primary aromatic amines.

(3) Dissolve 0.01 g of Triamterene in 100 mL of acetic acid (100), and to 10 mL of the solution add water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) Heavy metals $\langle 1.07 \rangle$ —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 $\langle 1.11 \rangle$ —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. To 2 mL of this solution add methanol to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample 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 $\langle 2.03 \rangle$. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ammonia solution (28) and methanol (9:1:1) to a distance of about 10 cm, and airdry the plate. Examine the plate under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution. JP XVI

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.10% (1 g).

Assay Weigh accurately about 0.15 g of Triamterene, previously dried, and dissolve in 100 mL of acetic acid (100) by warming. Titrate $\langle 2.50 \rangle$ with 0.05 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS = $12.66 \text{ mg of } C_{12}H_{11}N_7$

Containers and storage Containers-Well-closed containers.

Trichlormethiazide

トリクロルメチアジド



C₈H₈Cl₃N₃O₄S₂: 380.66

(3*RS*)-6-Chloro-3-dichloromethyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide [*133-67-5*]

Trichlormethiazide, when dried, contains not less than 97.5% and not more than 102.0% of $C_8H_8Cl_3N_3O_4S_2$.

Description Trichlormethiazide occurs as a white powder.

It is freely soluble in N, N-dimethylformamide and in acetone, slightly soluble in acetonitrile and in ethanol (95), and practically insoluble in water.

A solution of Trichlormethiazide in acetone (1 in 50) shows no optical rotation.

Melting point: about 270°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Trichlormethiazide in ethanol (95) (3 in 250,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Trichlormethiazide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Trichlormethiazide as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Trichlormethiazide RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Trichlormethiazide as directed under Flame Coloration Test $\langle 1.04 \rangle$ (2): a green color appears.

Purity (1) Chloride $\langle 1.03 \rangle$ —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 solution 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 $\langle 1.14 \rangle$ —Dissolve 1.0 g of Trichlormethiazide 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 $\langle 1.07 \rangle$ —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) Arsenic $\langle 1.11 \rangle$ —Prepare the test solution with 0.6 g of Trichlormethiazide according to Method 5, using 20 mL of *N*,*N*-dimethylformamide, and perform the test (not more than 3.3 ppm).

(5) Related substances—Dissolve 25 mg of Trichlormethiazide in 50 mL of acetonitrile, and use the solution as the sample solution. Perform the test with 10 μ L of the sample solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ 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-chlorobenzene-1,3-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 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with phenylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

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

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).

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

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 - 10 10 - 20	$\begin{array}{c} 100\\ 100 \rightarrow 0 \end{array}$	$\begin{array}{c} 0\\ 0 \rightarrow 100 \end{array}$

Flow rate: 1.5 mL per minute.

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

System suitability-

Test for required detectability: To exactly 1 mL of the sample solution add acetonitrile to make exactly 50 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add acetonitrile to make exactly 20 mL. Confirm that the

peak area of trichlormethiazide obtained from $10 \,\mu\text{L}$ of this solution is equivalent to 3.5 to 6.5% of that from $10 \,\mu\text{L}$ of the solution for system suitability test.

System performance: To 5 mL of the solution for system suitability test add 5 mL of water, and warm in a water bath at 60°C for 30 minutes. When the procedure is run with 10 μ L 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 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 $10 \,\mu\text{L}$ of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of trichlormethiazide is not more than 2.0%.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 25 mg of Trichlormethiazide and Trichlormethiazide RS, previously dried, and dissolve separately in exactly 20 mL of the internal standard solution. To 1 mL of these solutions add acetonitrile to make 20 mL, and use these solutions as the sample solution and standard solution. Perform the test with $10 \,\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of trichlormethiazide to that of the internal standard.

Amount (mg) of $C_8H_8Cl_3N_3O_4S_2 = M_S \times Q_T/Q_S$

M_S: Amount (mg) of Trichlormethiazide RS

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 inside diameter and 15 cm in length, packed with phenylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about $25^{\circ}C$.

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 minutes.

System suitability-

System performance: When the procedure is run with 10 μ L 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 $10 \,\mu\text{L}$ 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.

Trichlormethiazide Tablets

トリクロルメチアジド錠

Trichlormethiazide Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$: 380.66).

Method of preparation Prepare as directed under Tablets, with Trichlormethiazide.

Identification To an amount of pulverized Trichlormethiazide Tablets, equivalent to 4 mg of Trichlormethiazide according to the labeled amount, add 10 mL of acetone, shake vigorously for 5 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 4 mg of Trichlormethiazide RS in 10 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 5 μ L each of the sample 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 methanol (10:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spots from the sample solution and the standard solution show the same Rf value.

Purity Related substances-Pulverize a suitable amount of Trichlormethiazide Tablets in an agate mortar. Take an amount of the powder, equivalent to 10 mg of Trichlormethiazide according to the labeled amount, add 20 mL of acetonitrile, shake vigorously for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with $10 \,\mu$ L of the sample solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following 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 4amino-6-chlorobenzene-1,3-disulfoneamide, having the relative retention time of about 0.3 with respect to trichlormethiazide, is not more than 4.0%, and the total amount of the peaks other than trichlormethiazide is not more than 5.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-length: 268 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with phenylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

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

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).

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

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 - 10 10 - 20	$\begin{array}{c} 100\\ 100 \rightarrow 0 \end{array}$	$\begin{array}{c} 0\\ 0 \rightarrow 100 \end{array}$

Flow rate: 1.5 mL per minute.

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

System suitability—

Test for required detectability: Dissolve 25 mg of Trichlormethiazide in 50 mL of acetonitrile. To 1 mL of this solution add acetonitrile to make 50 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of trichlormethiazide obtained from $10 \,\mu$ L of this solution is equivalent to 3.5 to 6.5% of that obtained from $10 \,\mu$ L of the solution for system suitability test.

System performance: To 5 mL of the solution for system suitability test add 5 mL of water, and warm in a water bath of 60°C for 30 minutes. When the procedure is run with 10 μ L 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 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 $10 \,\mu\text{L}$ of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of trichlormethiazide is not more than 2.0%.

Uniformity of dosage units $\langle 6.02 \rangle$ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Trichlormethiazide Tablets add 5 mL of diluted phosphoric acid (1 in 50) to disintegrate. Add exactly an amount of the internal standard solution, equivalent to 10 mL per 2 mg of trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$), add acetonitrile to make 25 mL, shake vigorously for 15 minutes, and centrifuge. To an amount of the supernatant liquid add the mobile phase to make a solution so that it contains about $40 \mu g$ of trichlormethiazide (C₈H₈Cl₃N₃O₄S₂) in each mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Trichlormethiazide RS, previously dried at 105°C for 3 hours, and dissolve in acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add 10 mL of acetonitrile and 5 mL of diluted phosphoric acid (1 in 50), and use this solution as the standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the conditions described in the Assay, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of trichlormethiazide to that of the internal standard.

Amount (mg) of trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$) = $M_S \times Q_T/Q_S \times C \times 1/20$ $M_{\rm S}$: Amount (mg) of Trichlormethiazide RS

C: Labeled amount (mg) of trichlormethiazide $(C_8H_8Cl_3N_3O_4S_2)$ in 1 tablet

Internal standard solution—A solution of 3-nitrophenol in acetonitrile (1 in 5000).

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Trichlormethiazide Tablets is not less than 75%.

Start the test with 1 tablet of Trichlormethiazide Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding $0.45 \,\mu\text{m}$. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add diluted phosphoric acid (1 in 50) to make exactly V' mL so that each mL contains about $1.1 \,\mu g$ of trichlormethiazide (C₈H₈Cl₃N₃O₄S₂) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Trichlormethiazide RS, previously dried at 105°C for 3 hours, and dissolve in acetonitrile to make exactly 200 mL. Pipet 2 mL of this solution, add diluted phosphoric acid (1 in 50) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 40 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_{Ta} and A_{Sa} , of trichlormethiazide obtained with the sample solution and standard solution, and the area, A_{Tb} , of the peak, having the relative retention time of about 0.3 to trichlormethiazide, obtained with the sample solution.

Dissolution rate (%) with respect to the labeled amount of trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$)

- $= M_{\rm S} \times (A_{\rm Ta} + 0.95A_{\rm Tb})/A_{\rm Sa} \times V'/V \times 1/C \times 9/2$
- $M_{\rm S}$: Amount (mg) of Trichlormethiazide RS
- C: Labeled amount (mg) of trichlormethiazide $(C_8H_8Cl_3N_3O_4S_2)$ in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability-

System performance: Dissolve 25 mg of Trichlormethiazide in 50 mL of acetonitrile. To 1 mL of this solution add acetonitrile to make 50 mL. To 5 mL of this solution add 5 mL of water, and heat at 60°C in a water bath for 30 minutes. After cooling, when the procedure is run with $10 \,\mu$ L of this solution 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 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 6 times with $40 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trichlormethiazide is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Trichlormethiazide Tablets, and pulverize the tablets in

an agate mortar. Weigh accurately an amount of the powder, equivalent to about 2 mg of trichlormethiazide (C₈H₈Cl₃N₃O₄S₂), add 5 mL of diluted phosphoric acid (1 in 50) and exactly 10 mL of the internal standard solution, add 10 mL of acetonitrile, shake vigorously for 15 minutes, and centrifuge. To 2 mL of the supernatant liquid add 2 mL of the mobile phase, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of Trichlormethiazide RS, previously dried at 105°C for 3 hours, and dissolve in acetonitrile to make exactly 200 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add 10 mL of acetonitrile and 5 mL of diluted phosphoric acid (1 in 50), and use this solution as the standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of trichlormethiazide to that of the internal standard.

Amount (mg) of trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$) = $M_5 \times Q_T/Q_5 \times 1/20$

 $M_{\rm S}$: Amount (mg) of Trichlormethiazide RS

Internal standard solution—A solution of 3-nitrophenol in acetonitrile (1 in 5000).

Operating conditions-

Detector: An ultraviolet absorption photometer (wavelength: 268 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with phenylsilanized silica gel for liquid chromatography (5 μ m 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 minutes.

System suitability-

System performance: When the procedure is run with 20 μ L 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 $20 \,\mu\text{L}$ 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—Tight containers.

Trichomycin





Trichomycin A

33-(3-Amino-3,6-dideoxy- β -D-mannopyranosyloxy)-17-[6-(4-aminophenyl)-4-hydroxy-1-methyl-6-oxohexyl]-1,3,5,9,11,37-hexahydroxy-18-methyl-13,15-dioxo-16,39dioxabicyclo[33.3.1]nonatriaconta-19,21,23,25,27,29,31heptaene-36-carboxylic acid

[12698-99-6]

Trichomycin B

33-(3-Amino-3,6-dideoxy- β -D-mannopyranosyloxy)-17-[6-(4-aminophenyl)-4-hydroxy-1-methyl-6-oxohexyl]-1,3,5,7,9,37-hexahydroxy-18-methyl-13,15-dioxo-16,39dioxabicyclo[33.3.1]nonatriaconta-19,21,23,25,27,29,31heptaene-36-carboxylic acid

[12699-00-2]

[1394-02-1, Trichomycin]

Trichomycin is a mixture of polyene macrolide substances having antifungal and antiprotozoal activities produced by the growth of *Streptomyces hachijoensis*.

It contains not less than 7000 Units per mg, calculated on the dried basis. The potency of Trichomycin is expressed as unit based on the amount of trichomycin. One unit of Trichomycin is equivalent to $0.05 \ \mu g$ of trichomycin.

Description Trichomycin occurs as a yellow to yellowbrown powder.

It is practically insoluble in water, in ethanol (99.5) and in tetrahydrofuran.

It dissolves in dilute sodium hydroxide TS.

It is hygroscopic.

Identification (1) To 2 mg of Trichomycin add 2 mL of sulfuric acid: a blue color appears, and the color is changed to a blue-purple after allowing to stand.

(2) Dissolve 1 mg of Trichomycin in 50 mL of a solution of sodium hydroxide (1 in 200). Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: it exhibits maxima between 359 nm and 365 nm, between 378 nm and 384 nm, and between 400 nm and 406 nm.

Content ratio of the active principle Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve about 10 mg of Trichomycin in 50 mL of a mixture of tetrahydrofuran for liquid chromatography and water (3:1), and use this solution as the sample solution. Perform the test with 5μ L of the sample solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, determine the peak areas by the automatic integration method, and calculate the amount of trichomycin A and trichomycin B by the area percentage

method: the amount of trichomycin A is between 20% and 40%, and that of trichomycin B is between 15% and 25%. The relative retention time of trichomycin B with respect to trichomycin A is about 1.2.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 360 nm).

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

Column temperature: A constant temperature of about $25^{\circ}C$.

Mobile phase: Dissolve 3.4 g of potassium dihydrogen phosphate and 1.7 g of sodium lauryl sulfate in a mixture of 600 mL of water and 400 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of trichomycin A is about 8 minutes.

Time span of measurement: About 4 times as long as the retention time of trichomycin A.

System suitability—

Test for required detectability: Measure 5 mL of the sample solution, add a mixture of tetrahydrofuran for liquid chromatography and water (3:1) 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, and add a mixture of tetrahydrofuran for liquid chromatography and water (3:1) to make exactly 30 mL. Confirm that the peak area of trichomycin A obtained from 5 μ L of the solution is equivalent to 12 to 22% of that from 5 μ L of the solution for system suitability test.

System performance: When the procedure is run with $5 \,\mu L$ of the solution for system suitability test under the above operating conditions, trichomycin A and trichomycin B 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 5 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of trichomycin A is not more than 2.0%.

Loss on drying $\langle 2.41 \rangle$ Not more than 5.0% (1 g, in vacuum, 60°C, 3 hours).

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately an amount of Trichomycin and Trichomycin RS, equivalent to about 150,000 units, dissolve them separately in a mixture of tetrahydrofuran for liquid chromatography and water (3:1) to make exactly 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of trichomycin.

Amount (unit) of trichomycin = $M_{\rm S} \times A_{\rm T}/A_{\rm S}$

M_S: Amount (unit) of Trichomycin RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 360 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with silica gel for liquid chromatography (10 μ m in particle diameter).

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

Mobile phase: Dissolve 15 g of ammonium acetate in 120 mL of water, and add 1000 mL of acetonitrile for liquid chromatography and 700 mL of methanol.

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

System suitability-

System performance: Dissolve 5 mg of Trichomycin and 1 mg of berberine chloride in 100 mL of a mixture of tetrahydrofuran for liquid chromatography and water (3:1). When the procedure is run with $20 \,\mu$ L of this solution under the above operating conditions, berberine and trichomycin 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 $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trichomycin is not more than 2.0%.

Containers and storage Containers—Tight containers. Storage—Light-resistant, and in a cold place.

Triclofos Sodium

Monosodium Trichloroethyl Phosphate

トリクロホスナトリウム

C₂H₃Cl₃NaO₄P: 251.37

Monosodium 2,2,2-trichloroethyl monohydrogenphosphate [7246-20-0]

Triclofos Sodium, when dried, contains not less than 97.0% and not more than 102.0% of $C_2H_3Cl_3NaO_4P$, and not less than 41.0% and not more than 43.2% of chlorine (Cl: 35.45).

Description Triclofos Sodium is a white, crystalline powder.

It is freely soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

It is hygroscopic.

Identification (1) Determine the infrared absorption spectrum of Triclofos Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) To 0.5 g of Triclofos Sodium add 10 mL of nitric acid, evaporate on a water bath to dryness, and ignite further over a flame. Dissolve the residue in 5 mL of water, and filter it necessary: the filtrate responds to Qualitative Tests $\langle 1.09 \rangle$ for sodium salt.

(3) To 0.1 g of Triclofos Sodium add 1 g of anhydrous sodium carbonate, and heat for 10 minutes. After cooling, dissolve the residue in 40 mL of water, filter if necessary, and render the filtrate acidic with dilute nitric acid: the solution responds to the Qualitative Tests $\langle 1.09 \rangle$ (2) for chloride.

The remainder of the filtrate responds to the Qualitative Tests $\langle 1.09 \rangle$ (1) for chloride and to the Qualitative Tests $\langle 1.09 \rangle$ for phosphate.

pH $\langle 2.54 \rangle$ Dissolve 1.0 g of Triclofos Sodium in 50 mL of water: the pH of this solution is between 3.0 and 4.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Triclofos Sodium in 50 mL of water: the solution is clear and colorless.

(2) Chloride $\langle 1.03 \rangle$ —Perform the test with 0.20 g of Triclofos Sodium. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.178%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Triclofos 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).

(4) Arsenic $\langle 1.11 \rangle$ —Prepare the test solution with 1.0 g of Triclofos Sodium according to Method 1, and perform the test (not more than 2 ppm).

(5) Free phosphoric acid—Weigh accurately about 0.3 g of Triclofos Sodium, previously dried, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Pipet 5 mL each of the sample solution and Standard Phosphoric Acid Solution, add 2.5 mL of hexaammonium heptamolybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS, shake, add water to make exactly 25 mL, and allow to stand at 20°C for 30 minutes. Perform the test with these solutions, using a solution obtained in the same manner with 5 mL of water as the blank, as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$. Determine the absorbances, $A_{\rm T}$ and $A_{\rm S}$, of each solution from the sample solution and Standard Phosphoric Acid Solution at 740 nm: the content of the free phosphoric acid is not more than 1.0%.

Content (%) of the free phosphoric acid (H₃PO₄) = $1/M \times A_T/A_S \times 258.0$

M: Amount (mg) of the sample

Loss on drying $\langle 2.41 \rangle$ Not more than 5.0% (1 g, in vacuum, 100°C, 3 hours).

Assay (1) Triclofos sodium-Weigh accurately about 0.2 g of Triclofos Sodium, previously dried, place in a Kjeldhal flask, add 2 mL of sulfuric acid and 2.5 mL of nitric acid, and heat until brown gas are not evolved. After cooling, add 1 mL of nitric acid, heat until white fumes are produced, and cool. Repeat this procedure until the solution becomes colorless. Transfer this solution to a flask using 150 mL of water, add 50 mL of molybdenum (III) oxide-citric acid TS, heat gently to boil, add gradually 25 mL of quinoline TS with stirring, and heat on a water bath for 5 minutes. After cooling, filter the precipitate, and wash repeatedly with water until the washing does not indicate acidity. Transfer the precipitate to a flask using 100 mL of water, add exactly 50 mL of 0.5 mol/L sodium hydroxide VS, dissolve, and titrate <2.50> with 0.5 mol/L hydrochloric acid VS until the color of the solution changes from purple to yellow (indicator: 3 drops of phenolphthalein-thymol blue TS). Perform a blank determination.

> Each mL of 0.5 mol/L sodium hydroxide VS = 4.834 mg of C₂H₃Cl₃NaO₄P

(2) Chlorine—Weigh accurately about 10 mg of Triclofos Sodium, previously dried, perform the test according to the procedure of determination for chlorine as directed under Oxygen Flask Combustion Method $\langle 1.06 \rangle$, using 1 mL of sodium hydroxide TS and 20 mL of water as the absorbing liquid.

Containers and storage Containers—Tight containers.

Triclofos Sodium Syrup

Monosodium Trichloroethyl Phosphate Syrup

トリクロホスナトリウムシロップ

Triclofos Sodium Syrup contains not less than 90.0% and not more than 110.0% of the labeled amount of triclofos sodium (C₂H₃Cl₃NaO₄P: 251.37).

Method of preparation Prepare as directed under Syrups, with Triclofos Sodium.

Identification (1) Weigh a portion of Triclofos Sodium Syrup, equivalent to 0.25 g of Triclofos Sodium according to the labeled amount, add 40 mL of water, shake well, add 5 mL of diluted sulfuric acid (3 in 50), and extract with 25 mL of 3-methyl-1-butanol. Take 5 mL of the extract, evaporate on a water bath to dryness, and add 1 mL of diluted sulfuric acid (1 in 2) and 1 mL of a solution of potassium permanganate (1 in 20) to the residue. Heat in a water bath for 5 minutes, add 7 mL of water, and then add a solution of oxalic acid dihydrate (1 in 20) until the color of the solution disappears. To 1 mL of this solution add 1 mL of pyridine and 1 mL of a solution of sodium hydroxide (1 in 5), and heat in a water bath, while shaking, for 1 minute: a light red color develops in the pyridine layer.

(2) Take 10 mL of the extract obtained in (1), evaporate on a water bath to dryness, add 1 g of anhydrous sodium carbonate to the residue, and heat for 10 minutes. After cooling, dissolve the residue in 40 mL of water, filter if necessary, and render the filtrate acidic with dilute nitric acid: the solution responds to the Qualitative Tests $\langle 1.09 \rangle$ (2) for chloride. The remainder of the filtrate responds to the Qualitative Tests $\langle 1.09 \rangle$ (1) for chloride and to the Qualitative Tests $\langle 1.09 \rangle$ for phosphate.

pH <2.54> 6.0 - 6.5

Assay Weigh accurately a portion of Triclofos Sodium Syrup, equivalent to 0.13 g of Triclofos Sodium according to the labeled amount, add 15 mL of water, 1 mL of sodium hydroxide TS and 15 mL of diethyl ether, shake for 1 minute, and separate the water layer. Wash the diethyl ether layer with 1 mL of water, and combine the washing with above water layer. To this solution add 2.5 mL of diluted sulfuric acid (3 in 50), and extract with four 10-mL portions of 3-methyl-1-butanol. Combine the 3-methyl-1-butanol extracts, and add 3-methyl-1-butanol to make exactly 50 mL. Measure exactly 10 mL each of this solution, and dilute with potassium hydroxide-ethanol TS. Place in a glass ampule, fire-seal, mix, and heat at 120°C for 2 hours in an autoclave. After cooling, transfer the contents to a flask, add 20 mL of diluted nitric acid (63 in 500) and exactly 25 mL of 0.02 mol/L silver nitrate VS, shake well, and titrate <2.50> the excess silver nitrate with 0.02 mol/L ammonium thiocyanate VS (indicator: 2 to 3 drops of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.02 mol/L silver nitrate VS = 1.676 mg of C₂H₃Cl₃NaO₄P

Containers and storage Containers—Tight containers. Storage—In a cold place.

Trihexyphenidyl Hydrochloride

トリヘキシフェニジル塩酸塩



C₂₀H₃₁NO.HCl: 337.93

(1*RS*)-1-Cyclohexyl-1-phenyl-3-(piperidin-1-yl)propan-1-ol monohydrochloride [52-49-3]

and enantiomer

Trihexyphenidyl Hydrochloride, when dried, con-

tains not less than 98.5% of $C_{20}H_{31}$ NO.HCl.

Description Trihexyphenidyl Hydrochloride occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It 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 diethyl ether.

Melting point: about 250°C (with decomposition).

Identification (1) Dissolve 1 g of Trihexyphenidyl Hydrochloride in 100 mL of water by warming, and cool. Use this solution as the sample solution. To 5 mL of the sample solution add 1 mL of a solution of 2,4,6-trinitrophenol in chloroform (1 in 50), and shake vigorously: a yellow precipitate is formed.

(2) To 20 mL of the sample solution obtained in (1) add 2 mL of sodium hydroxide TS: a white precipitate is formed. Collect the precipitate, wash with a small amount of water, recrystallize from methanol, and dry in a desiccator (in vacuum, silica gel) for 2 hours: the crystals so obtained melt $\langle 2.60 \rangle$ between 113°C and 117°C.

(3) The sample solution obtained in (1) responds to the Qualitative Tests $\langle 1.09 \rangle$ (2) for chloride.

pH $\langle 2.54 \rangle$ 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 $\langle 1.07 \rangle$ —Dissolve 1.5 g of Trihexyphenidyl Hydrochloride in 60 mL of water by warming on 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) Piperidylpropiophenone—Dissolve 0.10 g of Trihexyphenidyl Hydrochloride in 40 mL of water and 1 mL of 1 mol/L hydrochloric acid VS by warming, cool, and add water to make 100 mL. Determine the absorbance of this solution at 247 nm as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: the absorbance is not more than 0.50.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid-dioxane VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid-dioxane VS = 33.79 mg of C₂₀H₃₁NO.HCl

Containers and storage Containers—Tight containers.

Trihexyphenidyl Hydrochloride Tablets

トリヘキシフェニジル塩酸塩錠

Trihexyphenidyl Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO.HCl:$ 337.93).

Method of preparation Prepare as directed under Tablets, with Trihexyphenidyl Hydrochloride.

Identification (1) Weigh a quantity 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 sample solution. With 5 mL of the sample solution, proceed as directed in the Identification (1) under Trihexyphenidyl Hydrochloride.

(2) Shake a quantity of powdered Trihexyphenidyl Hydrochloride Tablets, equivalent to 0.01 g of Trihexyphenidyl Hydrochloride according to the labeled amount, with 5 mL of chloroform, filter, and use the filtrate as the sample solution. Dissolve 0.02 g of Trihexyphenidyl Hydrochloride RS in 10 mL of chloroform, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 10 μ L each of the sample solution and 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 hydrogen hexachloroplatinate (IV)-potassium iodide TS on the plate: the spots from the sample solution and the standard solution show a blue-purple color and the same *R* f value.

(3) The sample solution obtained in (1) responds to the Qualitative Tests $\langle 1.09 \rangle$ (2) for chloride.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 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 on a water bath with occasional shaking for 10 minutes. Cool, add 2 mL of methannol, and add water to make exactly V mL of the solution contains about $20 \,\mu g$ of trihexyphenidyl hydrochloride (C₂₀H₃₁NO.HCl) per ml. Centrifuge, if necessary, and use the supernatant liquid as the sample solution. Separately, dissolve about 20 mg of Trihexyphenidyl Hydrochloride RS (determine previously its loss on drying <2.41> in the same conditions as Trihexyphenidyl Hydrochloride) in methanol to make exactly 20 mL. Pipet 2 mL of this solution, and 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 sample solution and standard solution, transfer to glass-stoppered centrifuge tubes, add exactly 10 mL of bromocresol purple-dipotassium hydrogenphosphate-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, $A_{\rm T}$ and $A_{\rm S}$, of the subsequent solutions of the sample solution and standard solution at 408 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, respectively.

> Amount (mg) of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO.HCl$) = $M_S \times A_T/A_S \times V/1000$

 $M_{\rm S}$: Amount (mg) of Trihexyphenidyl Hydrochlochloride RS, calculated on the dried basis

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Trihexyphenidyl Hydrochloride Tablets is not less than 70%.

Start the test with 1 tablet of Trihexyphenidyl Hydrochloride Tablets, withdraw not less than 30 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding $0.8 \,\mu\text{m}$. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V'mL so that each mL contains about $2.2 \,\mu g$ of trihexyphenidyl hydrochloride (C20H31NO.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Trihexyphenidyl Hydrochloride RS, previously dried at 105°C for 3 hours, and dissolve in the dissolution medium to make exactly 100 mL. Pipet 2 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Pipet 20 mL each of the sample solution, the standard solution and the dissolution medium add exactly 1 mL of diluted acetic acid (31) (1 in 10), and immediately add 5 mL of bromocresol greensodium 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, $A_{\rm T}$, $A_{\rm S}$ and $A_{\rm B}$, of these dichloromethane layers at 415 nm as directed under Ultravioletvisible Spectrophotometry $\langle 2.24 \rangle$, using dichloromethane as

the blank.

Dissolution rate (%) with respect to the labeled amount of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO.HCl$)

 $= M_{\rm S} \times (A_{\rm T} - A_{\rm B})/(A_{\rm S} - A_{\rm B}) \times V'/V \times 1/C \times 18$

 $M_{\rm S}$: Amount (mg) of Trihexyphenidyl Hydrochloride RS C: Labeled amount (mg) of trihexyphenidyl hydrochloride (C₂₀H₃₁NO.HCl) in 1 tablet

Assay Weigh accurately and powder not less than 20 Trihexyphenidyl Hydrochloride Tablets. Weigh accurately a portion of the powder, equivalent to about 5 mg of trihexyphenidyl hydrochloride (C₂₀H₃₁NO.HCl), dissolve in 2 mL of dilute hydrochloric acid and 60 mL of water by warming on a water bath for 10 minutes with occasional shaking. After cooling, add 2 mL of methanol and water to make exactly 100 mL, and use this solution as the sample solution. Dissolve about 50 mg of Trihexyphenidyl Hydrochloride RS (determine previously its loss on drying $\langle 2.41 \rangle$ in the same conditions as Trihexyphenidyl Hydrochloride), weighed accurately, in methanol, add 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 sample solution and standard solution into glass-stoppered centrifuge tubes, add exactly 10 mL each of bromocresol purple-dipotassium hydrogenphosphate-citric acid TS and 15 mL each of chloroform, stopper tightly, shake thoroughly, and centrifuge. Pipet 10 mL each of the chloroform layers, and add chloroform to make exactly 50 mL. Determine the absorbances, $A_{\rm T}$ and $A_{\rm S}$, of the subsequent solutions of the sample solution and standard solution at 408 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, respectively.

> Amount (mg) of trihexyphenidyl hydrochloride ($C_{20}H_{31}$ NO.HCl) = $M_S \times A_T/A_S \times 1/10$

 $M_{\rm S}$: Amount (mg) of Trihexyphenidyl Hydrochloride RS, calculated on the dried basis

Containers and storage Containers-Tight containers.

Trimebutine Maleate

トリメブチンマレイン酸塩



C₂₂H₂₉NO₅.C₄H₄O₄: 503.54 (2*RS*)-2-Dimethylamino-2-phenylbutyl 3,4,5trimethoxybenzoate monomaleate [*34140-59-5*]

Trimebutine Maleate, when dried, contains not less than 98.5% and not more than 101.0% of $C_{22}H_{29}NO_5.C_4H_4O_4$.

Description Trimebutine Maleate occurs as white, crystals or crystalline powder.

It is freely soluble in N, N-dimethylformamide and in acetic acid (100), soluble in acetonitrile, and slightly soluble in water and in ethanol (99.5).

It dissolves in 0.01 mol/L hydrochloric acid TS.

A solution of it in N,N-dimethylformamide (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Trimebutine Maleate in 0.01 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Trimebutine Maleate as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 131 – 135°C

Purity (1) Heavy metals $\langle 1.07 \rangle$ —Proceed with 2.0 g of Trimebutine 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).

(2) Arsenic $\langle 1.11 \rangle$ —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 sample solution. Pipet 1 mL of the sample 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 exactly $20 \,\mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than maleic acid and trimebutine from the sample solution is not larger than 1/2 times the peak area of trimebutine from the standard solution, and the total area of the peaks other than maleic acid and trimebutine 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 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

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

Mobile phase: To 650 mL of diluted perchloric acid (17 in 20,000), previously adjusted the pH to 3.0 with a solution of ammonium acetate (1 in 1000), add 1 g of sodium 1-pentanesulfonate to dissolve. 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.

Time span of measurement: About 2 times as long as the retention time of trimebutine beginning after the peak of

maleic acid.

System suitability-

Test for required detectability: Measure exactly 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 μ L of this solution is equivalent to 20 to 30% of that from 20 μ L of 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 TS and acetonitrile (13:7). When the procedure is run with 20μ L 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 $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trimebutine is not more than 5%.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 $\langle 2.50 \rangle$ 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 crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 50.35 mg of C₂₂H₂₉NO₅.C₄H₄O₄

Containers and storage Containers—Well-closed containers.

Trimetazidine Hydrochloride

トリメタジジン塩酸塩



C₁₄H₂₂N₂O₃.2HCl: 339.26 1-(2,3,4-Trimethoxybenzyl)piperazine dihydrochloride [*13171-25-0*]

Trimetazidine Hydrochloride contains not less than 98.0% and not more than 101.0% of $C_{14}H_{22}N_2O_3$. 2HCl, calculated on the anhydrous basis.

Description Trimetazidine Hydrochloride occurs as a white, crystalline powder.

It is very soluble in water and in formic acid, sparingly soluble in methanol, and slightly soluble in ethanol (99.5).

The pH of a solution of Trimetazidine Hydrochloride (1 in 20) is between 2.3 and 3.3.

Melting point: about 227°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Trimetazidine Hydrochloride in 0.1 mol/L hy-

drochloric acid TS (1 in 6250) as directed under Ultravioletvisible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Trimetazidine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Trimetazidine Hydrochloride (1 in 50) responds to the Qualitative Tests $\langle 1.09 \rangle$ for chloride.

Purity (1) Heavy metals $\langle 1.07 \rangle$ —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 sample solution. Pipet 2 mL of the sample 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 exactly $10 \,\mu L$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than trimetazidine obtained from the sample solution is not larger than 1.5 times that of trimetazidine from the standard solution, and the total area of the peaks other than trimetazidine from the sample 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 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

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

Mobile phase A: Dissolve 2.87 g of sodium 1-heptanesulfonate in water to make 1000 mL, and adjust the pH to 3.0 with diluted phosphoric acid (1 in 10). Mix 3 volumes of this solution and 2 volumes of methanol.

Mobile phase B: Methanol.

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

Time after injection	Mobile phase A	Mobile phase B
of sample (min)	(vol%)	(vol%)
0 - 50	$95 \rightarrow 75$	$5 \rightarrow 25$

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 $10 \,\mu$ L of

this solution is equivalent to 18 to 32% of that from $10 \,\mu L$ of the standard solution.

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of trimetazidine are not less than 15,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ 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 $\langle 2.48 \rangle$ Not more than 1.5% (2 g, volumetric titration, direct titration).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.12 g of Trimetazidine Hydrochloride, dissolve in 5 mL of formic acid, add exactly 15 mL of 0.1 mol/L perchloric acid VS, and heat at 90 – 100°C for 30 minutes. After cooling, add 45 mL of acetic acid (100), and titrate $\langle 2.50 \rangle$ the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L perchloric acid VS = $16.96 \text{ mg of } C_{14}H_{22}N_2O_3.2\text{HCl}$

Containers and storage Containers—Tight containers.

Trimetazidine Hydrochloride Tablets

トリメタジジン塩酸塩錠

Trimetazidine Hydrochloride Tablets contain not less than 94.0% and not more than 106.0% of the labeled amount of trimetazidine hydrochloride ($C_{14}H_{22}N_2O_3$.2HCl: 339.26).

Method of preparation Prepare as directed under Tablets, with Trimetazidine Hydrochloride.

Identification Shake a quantity of powdered Trimetazidine Hydrochloride Tablets, equivalent to 10 mg of Trimetazidine Hydrochloride according to the labeled amount, with 10 mL of a mixture of ethanol (95) and water (3:1), and filter. Evaporate the filtrate on a water bath, add 2 mL of water to the residue, and shake. To 1 mL of this solution add 1 mL of *p*-benzoquinone TS, boil gently for 2 to 3 minutes, and cool: a red color develops.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Trimetazidine Hydrochloride Tablets add 15 mL of a mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1) to disintegrate the tablet, and treat with ultrasonic waves for 10 minutes. Shake the solution for 10 minutes, and add the mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1) to make exactly 20 mL. Centrifuge, pipet V mL of the supernatant liquid, equivalent to about 0.75 mg of trimetazidine hydrochloride (C₁₄H₂₂N₂O₃.2HCl), add exactly 2.5 mL of the internal

standard solution, add 0.1 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of trimetazidine hydrochloride for assay (separately determine the water $\langle 2.48 \rangle$ in the same manner as Trimetazidine Hydrochloride), and dissolve in the mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1) to make exactly 200 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and 0.1 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay.

Amount (mg) of trimetazidine hydrochloride ($C_{14}H_{22}N_2O_3.2HCl$) = $M_S \times Q_T/Q_S \times 1/2V$

 $M_{\rm S}$: Amount (mg) of trimetazidine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of parahydroxybenzoic acid in the mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1) (7 in 40,000).

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Trimetazidine Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Trimetazidine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding $0.45 \,\mu\text{m}$. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, and add water to make exactly V' mL so that each mL contains about $3.3 \,\mu g$ of trimetazidine hydrochloride (C14H22N2O3.2HCl) according to the labeled amount. Pipet 3 mL of this solution, add exactly 3 mL of 0.1 mol/L hydrochloric acid TS, and use this solution as the sample solution. Separately, weigh accurately about 17 mg of trimetazidine hydrochloride for assay (separately determine the water $\langle 2.48 \rangle$ in the same manner as Trimetazidine Hydrochloride), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, and add water to make exactly 25 mL. Pipet 3 mL of this solution, add exactly 3 mL of 0.1 mol/L hydrochloric acid TS, and use this solution as the standard solution. Perform the test with exactly $50\,\mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of trimetazidine.

Dissolution rate (%) with respect to the labeled amount of trimetazidine hydrochloride ($C_{14}H_{22}N_2O_3.2HCl$) = $M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

- $M_{\rm S}$: Amount (mg) of trimetazidine hydrochloride for assay, calculated on the anhydrous basis
- C: Labeled amount (mg) of trimetazidine hydrochloride (C₁₄H₂₂N₂O₃.2HCl) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 50 μ L of the standard solution under the above operating con-

ditions, the number of theoretical plates and the symmetry factor of the peak of trimetazidine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with $50 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trimetazidine is not more than 1.5%.

Assay Weigh accurately not less than 20 tablets of Trimetazidine Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 3 mg of trimetazidine hydrochloride (C14H22N2O3.2HCl), add about 15 mL of a mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1), and treat with ultrasonic waves for 10 minutes. Then shake for 10 minutes, add the mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1) to make exactly 20 mL, and centrifuge. To exactly 5 mL of the supernatant liquid add exactly 5 mL of the internal standard solution and 0.1 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of trimetazidine hydrochloride for assay (separately determine the water $\langle 2.48 \rangle$ in the same manner as Trimetazidine Hydrochloride), and dissolve in the mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1) to make exactly 200 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution and 0.1 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the standard solution. Perform the test with $10\,\mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of trimetazidine to that of the internal standard.

> Amount (mg) of trimetazidine hydrochloride ($C_{14}H_{22}N_2O_3.2HCl$) = $M_S \times Q_T/Q_S \times 1/10$

 $M_{\rm S}$: Amount (mg) of trimetazidine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of parahydroxybenzoic acid in the mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1) (7 in 40,000).

Operating conditions—

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

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

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

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate TS, pH 3.0 and methanol (17:3).

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

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, trimetazidine 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 $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of trimetazidine to that of the internal stand-

ard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Trimethadione

トリメタジオン



C₆H₉NO₃: 143.14 3,5,5-Trimethyl-1,3-oxazolidine-2,4-dione [*127-48-0*]

Trimethadione, when dried, contains not less than 98.0% of C₆H₉NO₃.

Description Trimethadione occurs as white crystals or crystalline powder. It has a camphor-like odor.

It is very soluble in ethanol (95) and in chloroform, freely soluble in diethyl ether, and soluble in water.

Identification (1) To 5 mL of a solution of Trimethadione (1 in 50) add 2 mL of barium hydroxide TS: a precipitate is formed immediately.

(2) Determine the infrared absorption spectrum of a solution of Trimethadione in chloroform (1 in 50) as directed in the solution method under Infrared Spectrophotometry $\langle 2.25 \rangle$, using a 0.1-mm fixed sodium chloride cell, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Meting point <2.60> 45 – 47°C

Purity Heavy metals $\langle 1.07 \rangle$ —Proceed with 2.0 g of Trimethadione 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).

Loss on drying <2.41> Not more than 0.5% (1 g, silica gel, 6 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Trimethadione, previously dried, in a glass-stoppered conical flask, dissolve in 5 mL of ethanol (95), add exactly measured 50 mL of 0.1 mol/L sodium hydroxide VS, stopper, and allow to stand for 15 minutes with occasional shaking. Titrate $\langle 2.50 \rangle$ the excess sodium hydroxide with 0.1 mol/L hydrochloric acid VS (indicator: 4 drops of cresol red TS). Perform a blank determination.

> Each mL of 0.1 mol/L sodium hydroxide VS = $14.31 \text{ mg of } C_6H_9NO_3$

Containers and storage Containers—Tight containers. Storage—Not exceeding 30°C.

Trimethadione Tablets

トリメタジオン錠

Trimethadione Tabelts contain not less than 94.0% and not more than 106.0% of the labeled amount of trimethadione (C₆H₉NO₃: 143.14).

Method of preparation Prepare as directed under Tablets, with Trimethadione.

Identification (1) Weigh a portion of powdered Trimethadione Tablets, equivalent to 1 g of Trimethadione according to the labeled amount, add 10 mL of petroleum benzin, and shake frequently for 15 minutes. Decant, remove the petroleum benzin, add another 10 mL of petroleum benzin, and repeat the extraction in the same manner. To the residue add 25 mL of diethyl ether, allow to stand for 20 minutes with occasional shaking, filter, evaporate the filtrate at room temperature, and dry the residue in a desiccator (silica gel) for 6 hours: the residue melts $\langle 2.60 \rangle$ between 44°C and 47°C. Proceed with this residue as directed in the Identification (1) under Trimethadione.

(2) Determine the infrared absorption spectrum of a solution of the residue obtained in (1) in chloroform (1 in 50) in a 0.1-mm fixed sodium chloride cell, as directed in the solution method under Infrared Spectrophotometry $\langle 2.25 \rangle$: it exhibits absorption at the wave numbers of about 2960 cm⁻¹, 1814 cm⁻¹, 1735 cm⁻¹, 1445 cm⁻¹, 1394 cm⁻¹, 1290 cm⁻¹, 1100 cm⁻¹ and 1055 cm⁻¹.

Assay Weigh accurately and powder not less than 20 Trimethadione Tablets. Weigh accurately a portion of the powder, equivalent to about 1 g of trimethadione (C₆H₉NO₃), add 50 mL of ethanol (95), and boil gently for 15 minutes under a reflux condenser. Filter the warm ethanol (95) solution into a 100-mL volumetric flask through a glass filter (G4), and wash the residue with three 10-mL portions of warm ethanol (95). Combine the washings with the filtrate in the flask, cool, and add ethanol (95) to make exactly 100 mL. Pipet 25 mL of the solution into a glass-stoppered conical flask, add 25 mL of water and exactly 30 mL of 0.1 mol/L sodium hydroxide VS, stopper, allow to stand for 15 minutes with occasional shaking, and titrate <2.50> the excess sodium hydroxide with 0.1 mol/L hydrochloric acid VS (indicator: 4 drops of cresol red TS). Perform a blank determination.

> Each mL of 0.1 mol/L sodium hydroxide VS = $14.31 \text{ mg of } C_6H_9NO_3$

Containers and storage Containers—Tight containers. Storage—Not exceeding 30°C.

Trimetoquinol Hydrochloride Hydrate

Tretoquinol Hydrochloride

トリメトキノール塩酸塩水和物



C₁₉H₂₃NO₅.HCl.H₂O: 399.87 (1*S*)-1-(3,4,5-Trimethoxybenzyl)-1,2,3,4tetrahydroisoquinoline-6,7-diol monohydrochloride monohydrate [*18559-59-6*, anhydride]

Trimetoquinol Hydrochloride Hydrate contains not less than 98.5% and not more than 101.0% of trimetoquinol hydrochloride ($C_{19}H_{23}NO_5$.HCl: 381.85), calculated on the anhydrous basis.

Description Trimetoquinol Hydrochloride Hydrate occurs as white, crystals or crystalline powder.

It is freely soluble in methanol, and sparingly soluble in water and in ethanol (99.5).

Melting point: about 151° C (with decomposition, after drying in vacuum, 105° C, 4 hours).

Identification (1) Determine the absorption spectrum of a solution of Trimetoquinol Hydrochloride Hydrate in 0.01 mol/L hydrochloric acid TS (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Trimetoquinol Hydrochloride Hydrate as directed in the potassium chloride disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Trimetoquinol Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <1.09> (1) for chloride.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_D^{20}$: $-16 - -19^\circ$ (0.25 g, calculated on the anhydrous basis, water, after warming and cooling, 25 mL, 100 mm).

pH $\langle 2.54 \rangle$ 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 $\langle 1.14 \rangle$ —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 <1.07>—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 sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution, as directed under Liquid Chromatography <2.01> 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 trimetoquinol from the sample 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 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m 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, and filter through a membrane filter with pore size of $0.4 \,\mu\text{m}$. 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.

Time span of measurement: About twice as long as the retention time of trimetoquinol beginning after the solvent peak.

System suitability-

Test for required detection: 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 $20 \,\mu\text{L}$ of this solution is equivalent to 7 to 13% of that from $20 \,\mu\text{L}$ 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 μ L of this solution under the above operating conditions, procaine and trimetoquinol 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 $20 \,\mu\text{L}$ 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%.

Water $\langle 2.48 \rangle$ 3.5 – 5.5% (0.3 g, volumetric titration, direct titration).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g 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 $\langle 2.50 \rangle$ with 0.1 mol/L potassium hydroxide-ethanol VS (potentiometric titration). Calculate the consumed volume of 0.1 mol/L potassium hydroxide-

ethanol VS between the first inflection point and of the second inflection point.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS = 38.19 mg of C₁₉H₂₃NO₅.HCl

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Dental Triozinc Paste

歯科用トリオジンクパスタ

Dental Triozinc Paste consists of a powder containing Paraformaldehyde, Thymol, anhydrous zinc sulfate and Zinc Oxide, and a solution containing Cresol, Potash Soap and Glycerin. Suitable amounts of the two components are triturated before use.

Method of preparation

(1) The powder	
Paraformaldehyde, finely powdered	10 g
Thymol, finely powdered	3 g
Zinc Sulfate Hydrate	9 g
Zinc Oxide	82 g
To make about	100 g

Heat Zinc Sulfate Hydrate at about 250°C to obtain anhydrous zinc sulfate, cool, and pulverize to a fine powder. Mix homogeneously this powder with Thymol, Paraformalde-hyde, and Zinc Oxide.

(2) The solution		
Cresol		40 g
Potash Soap		40 g
Glycerin		20 g
Т	`o make	100 g

Dissolve Potash Soap in a mixture of Cresol and Glycerin.

Description The powder occurs as a fine, white powder, having a characteristic odor. The solution is a clear, yellow-brown to red-brown, viscous liquid, having the odor of cresol.

Containers and storage Containers—Tight containers.

Tropicamide





C₁₇H₂₀N₂O₂: 284.35 (2*RS*)-*N*-Ethyl-3-hydroxy-2-phenyl-*N*-(pyridin-4-ylmethyl)propanamide [*1508-75-4*]

Tropicamide, when dried, contains not less than 98.5% of $C_{17}H_{20}N_2O_2$.

Description Tropicamide occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in ethanol (95) and in chloroform, slightly soluble in water and in diethyl ether, and practically insoluble in petroleum ether.

It dissolves in dilute hydrochloric acid.

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 (V) in sulfuric acid, (1 in 200), and heat: a blue-purple color develops.

(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-dinitrobenzene, and heat on 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 develops.

Absorbance $\langle 2.24 \rangle = E_{1 \text{ cm}}^{1\%}$ (255 nm): 166 – 180 (after drying, 5 mg, 2 mol/L hydrochloric acid TS, 200 mL).

Melting point <2.60> 96 – 99°C

Purity (1) Chloride $\langle 1.03 \rangle$ —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 <1.07>—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 hydrogen carbonate TS, and shake: no blue color develops.

(4) Tropic acid—To 10 mg of Tropicamide add 5 mg of sodium borate and 7 drops of 4-dimethylaminobenzaldehyde TS, and heat in a water bath for 3 minutes. Cool in ice water, and add 5 mL of acetic anhydride: no red-purple
JP XVI

Loss on drying $\langle 2.41 \rangle$ Not more than 0.30% (1 g, in vacuum, silica gel, 24 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 28.44 mg of $C_{17}H_{20}N_2O_2$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Troxipide

トロキシピド



C₁₅H₂₂N₂O₄: 294.35 3,4,5-Trimethoxy-*N*-[(3*RS*)-piperidin-3-yl]benzamide [30751-05-4]

Troxipide, when dried, contains not less than 98.5% and not more than 101.0% of $C_{15}H_{22}N_2O_4$.

Description Troxipide occurs as a white, crystalline powder.

It is freely soluble in acetate (100), soluble in methanol, sparingly soluble in ethanol (99.5) and slightly soluble in water.

It dissolves in 0.1 mol/L hydrochloric acid TS.

A solution of Troxipide in 1 mol/L hydrochloric acid TS (1 in 5) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Troxipide in 0.1 mol/L hydrochloric acid TS (1 in 62,500) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Troxipide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Troxipide as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Troxipide RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 177 – 181°C

Purity (1) Chloride $\langle 1.03 \rangle$ —Dissolve 1.0 g of Troxipide in 30 mL of methanol, 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 as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 30 mL of methanol, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.009%).

(2) Heavy metals <1.07>—Moisten 2.0 mg of Troxipide with 1 mL of sulfuric acid, and gently heat until charred. After cooling, add 2 mL of nitric acid, carefully heat until white fumes are no longer evolved, and perform the test according to Method 2. Prepare the control solution as follows: evaporate 1 mL of sulfuric acid, 2 mL of nitric acid and 2 mL of hydrochloric acid on a water bath and then on a sand bath to dryness, and moisten the residue with 3 drops of hydrochloric acid. Proceed in the same manner for the preparation of the test solution, and add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 10 ppm).

(3) Related substances—Dissolve 0.20 g of Troxipide in 10 mL of methanol, and use this solution as the sample solution. Pipet 2 mL of the sample 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 these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample 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, ethyl acetate, water, hexane and ammonia water (28) (20:20:5:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the number of the spots other than the principal spot obtained from the sample solution is not more than three, and they are not more intense than the spot from the standard solution.

(4) Residual solvent—Being specified separately.

Loss on drying $\langle 2.41 \rangle$ Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Troxipide, previously dried, dissolve in 40 mL of acetic acid (100), and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 29.44 mg of $C_{15}H_{22}N_2O_4$

Containers and storage Containers—Tight containers.

Troxipide Fine Granules

トロキシピド細粒

Troxipide Fine Granules contain not less than 93.0% and not more than 107.0% of the labeled amount of troxipide ($C_{15}H_{22}N_2O_4$: 294.35).

Method of preparation Prepare as directed under Granules, with Troxipide.

Identification To a quantity of Troxipide Fine Granules, equivalent to 20 mg of Troxipide according to the labeled amount, add 100 mL of 0.1 mol/L hydrochloric acid TS, stir, and filter. To 4 mL of the filtrate add 0.1 mol/L hydro-

1542 Troxipide Tablets / Official Monographs

chloric acid TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: it exhibits a maximum between 256 nm and 260 nm.

Uniformity of dosage units $\langle 6.02 \rangle$ Perform the test according to the following method: Troxipide Fine Granules in single-unit containers meet the requirement of the Content uniformity test.

To the total amount of the content of 1 container of Troxipide Fine Granules, add 80 mL of 0.1 mol/L hydrochloric acid TS, stir for 10 minutes, and add 0.1 mol/L hydrochloric acid TS to make exactly VmL so that each mL contains about 1 mg of troxipide (C₁₅H₂₂N₂O₄). Centrifuge this solution, pipet 2 mL of the supernatant liquid, add exactly 3 mL of the internal standard solution, and water to make 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of troxipide (C₁₅H₂₂N₂O₄)
=
$$M_{\rm S} \times Q_{\rm T}/Q_{\rm S} \times V/25$$

M_S: Amount (mg) of Troxipide RS

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L hydrochloric acid TS (3 in 2000).

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 60 minutes of Troxipide Fine Granules is not less than 85%.

Weigh accurately an amount of Troxipide Fine Granules, equivalent to about 0.1 g of troxipide (C15H22N2O4) according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding $0.8 \,\mu\text{m}$. Discard the first $10 \,\text{mL}$ of the filtrate, pipet 4 mL of the subsequent filtrate, add water to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Troxipide RS, previously dried at 105°C for 2 hours, and dissolve in water to make exactly 200 mL. Pipet 4 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, $A_{\rm T}$ and $A_{\rm S}$, at 258 nm.

Dissolution rate (%) with respect to the labeled amount of troxipide ($C_{15}H_{22}N_2O_4$)

 $= M_{\rm S}/M_{\rm T} \times A_{\rm T}/A_{\rm S} \times 1/C \times 450$

 $M_{\rm S}$: Amount (mg) of Troxipide RS

 $M_{\rm T}$: Amount (mg) of sample

C: Labeled amount (mg) of troxipide ($C_{15}H_{22}N_2O_4$) in 1 g

Particle size <6.03> It meets the requirements of Fine granules.

Assay Weigh accurately an amount of Troxipide Fine Granules, equivalent to about 0.5 g of troxipide $(C_{15}H_{22}N_2O_4)$, add 200 mL of 0.1 mol/L hydrochloric acid TS, stir for 10 minutes, and add 0.1 mol/L hydrochloric acid TS to make exactly 250 mL. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add 0.1 mol/L hydrochloric acid TS to make exactly 10 mL. Pipet 2 mL of this solution,

add exactly 3 mL of the internal standard solution, and water to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Troxipide RS, previously dried at 105 °C for 2 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 25 mL. Pipet 2 mL of this solution, add exactly 3 mL of the internal standard solution, and water to make 100 mL, and use this solution as the standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of troxipide to that of the internal standard.

Amount (mg) of troxipide $(C_{15}H_{22}N_2O_4)$ = $M_S \times Q_T/Q_S \times 20$

 $M_{\rm S}$: Amount (mg) of Troxipide RS

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L hydrochloric acid TS (3 in 2000). *Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 258 nm).

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

Column temperature: A constant temperature of about 30°C.

Mobile phase: To diluted phosphoric acid (1 in 500) add diethylamine to adjust the pH to 3.0. To 1500 mL of this solution add 100 mL of methanol and 50 mL of tetrahydronfuran.

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

System suitability-

System performance: When the procedure is run with 20 μ L of standard solution under the above operating conditions, troxipide 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 $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of troxipide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Troxipide Tablets

トロキシピド錠

Troxipide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of troxipide ($C_{15}H_{22}N_2O_4$: 294.35).

Method of preparation Prepare as directed under Tablets, with Troxipide.

Identification Weigh accurately an amount of powdered Troxipide Tablets, equivalent to 0.1 g of Troxipide according to the labeled amount, add 250 mL of 0.1 mol/L hydrochloric acid TS, shake, and filter. To 4 mL of the filtrate 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 <2.24>: it exhibits maximum between 256 nm and 260 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Troxipide Tablets add 90 mL of 0.1 mol/L hydrochloric acid TS, shake well to disintegrate, shake for another 10 minutes, and add 0.1 mol/L hydrochloric acid TS to make exactly V mL so that each mL contains about 1 mg of troxipide ($C_{15}H_{22}N_2O_4$). Centrifuge this solution, pipet 2 mL of the supernatant liquid, add exactly 3 mL of the internal standard solution, add water to make 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of troxipide ($C_{15}H_{22}N_2O_4$) = $M_S \times Q_T/Q_S \times V/25$

 $M_{\rm S}$: Amount (mg) of Troxipide RS

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L hydrochloric acid TS (3 in 2000).

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Troxipide Tablets is not less than 70%.

Start the test with 1 tablet of Troxipide Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding $0.8 \,\mu\text{m}$. 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 $22 \mu g$ of troxipide (C₁₅H₂₂N₂O₄) according to the labeled amount, and use this solution as the sample solution. Separately weigh accurately about 20 mg of Troxipide RS, previously dried at 105°C for 2 hours, and dissolve in water to make exactly 200 mL. Pipet 4 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and determine the absorbances, $A_{\rm T}$ and $A_{\rm S}$, at 258 nm.

Dissolution rate (%) with respect to the labeled amount of troxipide ($C_{15}H_{22}N_2O_4$)

 $= M_{\rm S} \times A_{\rm T}/A_{\rm S} \times V'/V \times 1/C \times 90$

 $M_{\rm S}$: Amount (mg) of Troxipide RS

C: Labeled amount (mg) of Troxipide ($C_{15}H_{22}N_2O_4$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Troxipide Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 1 g of troxipide ($C_{15}H_{22}N_2O_4$), add 150 mL of 0.1 mol/L hydrochloric acid TS, shake for 30 minutes, add 0.1 mol/L hydrochloric acid TS to make exactly 250 mL. Centrifuge this solution, pipet 5 mL of the supernatant liquid, and add 0.1 mol/L hydrochloric acid TS to make exactly 20 mL. Pipet 2 mL of this solution, add exactly 3 mL of the internal standard solution and water to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Troxipide RS, previously dried at 105°C for 2 hours, and dissolve in 0.1 mol/L

hydrochloric acid TS to make exactly 25 mL. Pipet 2 mL of this solution, add exactly 3 mL of the internal standard solution and water to make 100 mL, and use this solution as the standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of troxipide to that of the internal standard.

> Amount (mg) of troxipide (C₁₅H₂₂N₂O₄) = $M_{\rm S} \times Q_{\rm T}/Q_{\rm S} \times 40$

 $M_{\rm S}$: Amount (mg) of Troxipide RS

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L hydrochloric acid TS (3 in 2000). *Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 258 nm).

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

Column temperature: A constant temperature of about 30° C.

Mobile phase: To 1500 mL of diluted phosphoric acid (1 in 500) add diethylamine to adjust the pH to 3.0. To 1500 mL of this solution add 100 mL of methanol and 50 mL of tetrahydronfuran.

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

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, troxipide 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 $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of troxipide to that of the internal standard is not more than 1.0%.

Containers and storage Containers-Tight containers.

L-Tryptophan

L-トリプトファン



C₁₁H₁₂N₂O₂: 204.23 (2S)-2-Amino-3-(indol-3-yl)propanoic acid [73-22-3]

L-Tryptophan, when dried, contains not less than 98.5% of $C_{11}H_{12}N_2O_2$.

Description L-Tryptophan occurs as white to yellowish white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in formic acid, slightly soluble in water,

and very slightly soluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

Identification Determine the infrared absorption spectrum of L-Tryptophan, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_D^{20}$: $-30.0 - -33.0^\circ$ 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 optical rotation of the solution in a 100-mm cell.

pH $\langle 2.54 \rangle$ Dissolve 1.0 g 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 $0.20 \text{ g of } \text{L-Tryp-tophan in 10 mL of 2 mol/L hydrochloric acid TS: the solution is clear.$

(2) Chloride $\langle 1.03 \rangle$ —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 $\langle 1.14 \rangle$ —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 $\langle 1.02 \rangle$ —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 $\langle 1.07 \rangle$ —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) Arsenic $\langle 1.11 \rangle$ —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 with this solution as the test solution (not more than 2 ppm).

(7) 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 sample solution. Pipet 1 mL of the sample 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 these solutions as directed under Thinlayer Chromatography $\langle 2.03 \rangle$. Spot 5 μ L each of the sample solution and 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 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 sample solution are not more intense than the spot from the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of L-Tryptophan, pre-

viously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 20.42 mg of $C_{11}H_{12}N_2O_2$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Tulobuterol Hydrochloride

C12H18CINO.HCl: 264.19

and enantiomer

(1*RS*)-1-(2-Chlorophenyl)-2-(1,1-dimethylethyl)aminoethanol monohydrochloride [56776-01-3]

Tulobuterol Hydrochloride, when dried, contains not less than 98.5% of $C_{12}H_{18}CINO.HCI$.

Description Tulobuterol Hydrochloride occurs as white crystals or crystalline powder.

It is very soluble in methanol, freely soluble in water, in ethanol (95) and in acetic acid (100), sparingly soluble in acetic anhydride, and very slightly soluble in diethyl ether.

A solution of Tulobuterol Hydrochloride (1 in 20) shows no optical rotation.

Melting point: about 163°C.

Identification (1) Determine the absorption spectrum of a solution of Tulobuterol Hydrochloride (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tulobuterol Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectro-photometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spctra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Tulobuterol Hydrochloride (1 in 20) responds to the Qualitative Tests $\langle 1.09 \rangle$ 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 $\langle 1.07 \rangle$ —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 sample solution. Pipet 1 mL of the sample solution, add 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 $\langle 2.03 \rangle$. Use a

plate 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 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. 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 starting point from the sample solution are not more intense than the spot from the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (0.5 g, in vacuum, 60°C, 4 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

> Each mL of 0.1 mol/L perchloric acid VS = $26.42 \text{ mg of } C_{12}H_{18}CINO.HCl$

Containers and storage Containers—Tight containers.

Turpentine Oil

Oleum Terebinthinae

テレビン油

Turpentine Oil is the essential oil distilled with steam from the wood or balsam of *Pinus* species (*Pinaceae*).

Description Turpentine Oil is a clear, colorless to pale yellow liquid. It has a characteristic odor and a pungent, bitter taste.

Turpentine Oil (1 mL) is miscible with 5 mL of ethanol (95) and this solution is neutral.

Refractive index <2.45> $n_{\rm D}^{20}$: 1.465 – 1.478

Specific gravity $\langle 1.13 \rangle$ d_{20}^{20} : 0.860 - 0.875

Purity (1) Foreign matter—Turpentine Oil has no offensive odor. Shake 5 mL of Turpentine Oil with 5 mL of a solution of potassium hydroxide (1 in 6): the aqueous layer does not show a yellow-brown to dark brown color.

(2) Hydrochloric acid-coloring substances—Shake 5 mL of Turpentine Oil with 5 mL of hydrochloric acid, and allow to stand for 5 minutes: the hydrochloric acid layer is light yellow and not brown in color.

(3) Mineral oil—Place 5 mL of Turpentine Oil in a Cassia flask, cool to a temperature not exceeding 15° C, add dropwise 25 mL of fuming sulfuric acid while shaking, warm between 60°C and 65°C for 10 minutes, and add sulfuric acid to raise the lower level of the oily layer to the graduated portion of the neck: not more than 0.1 mL of oil separates.

Distilling range $\langle 2.57 \rangle$ 150 – 170°C, not less than 90 vol%.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

L-Tyrosine

L-チロシン



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C<sub>9</sub>H<sub>11</sub>NO<sub>3</sub>: 181.19
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(2S)-2-Amino-3-(4-hydroxyphenyl)propanoic acid [60-18-4]

L-Tyrosine, when dried, contains not less than 99.0% and not more than 101.0% of $C_9H_{11}NO_3$.

Description L-Tyrosine occurs as white crystals or a crystalline powder.

It is freely soluble in formic acid, and practically insoluble in water and in ethanol (99.5).

It dissolves in dilute hydrochloric acid and in ammonia TS.

Identification (1) Determine the absorption spectrum of a solution of L-Tyrosine in 0.1 mol/L hydrochloric acid (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of L-Tyrosine as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_D^{20}$: $-10.5 - -12.5^\circ$ (after drying, 2.5 g, 1 mol/L hydrochloric acid TS, 50 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 1.0 g of L-Tyrosine in 20 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Chloride $\langle 1.03 \rangle$ —Dissolve 0.5 g of L-Tyrosine in 12 mL of dilute nitric acid and 20 mL of water, 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 VS add 12 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%).

(3) Sulfate $\langle 1.14 \rangle$ —Dissolve 0.6 g of L-Tyrosine in 5 mL of dilute hydrochloric acid, and add water to make 45 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.35 mL of 0.005 mol/L sulfuric acid VS add 5 mL of dilute hydrochloric acid and water to make 45 mL. To the test solution and the control solution add 5 mL of barium chloride TS (not more than 0.028%).

(4) Ammonium $\langle 1.02 \rangle$ —Perform the test with 0.25 g of L-Tyrosine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals $\langle 1.07 \rangle$ —Proceed with 1.0 g of L-Tyrosine 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).

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(6) Iron $\langle 1.10 \rangle$ —Prepare the test solution with 1.0 g of L-Tyrosine according to Method 3, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Dissolve 0.20 g of L-Tyrosine in 10 mL of diluted ammonia solution (28) (1 in 2), add water to make 20 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 10 mL, 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 under Thinlayer Chromatography $\langle 2.03 \rangle$. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Then develop with a mixture of 1-propanol and ammonia solution (28) (67:33) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in a mixture of methanol and acetic acid (100) (97:3) (1 in 100) on the plate, and then heat at 80°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.3% (1 g, 105°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.18 g of L-Tyrosine previously dried, dissolve in 6 mL of formic acid, add 50 mL of acetic acid (100), and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = $18.12 \text{ mg of } C_9H_{11}NO_3$

Containers and storage Containers—Tight containers.

Ubenimex

ウベニメクス



C₁₆H₂₄N₂O₄: 308.37 (2*S*)-2-[(2*S*,3*R*)-3-Amino-2-hydroxy-4-phenylbutanoylamino]-4-methylpentanoic acid [*58970-76-6*]

Ubenimex, when dried, contains not less than 98.5% and not more than 101.0% of $C_{16}H_{24}N_2O_4$.

Description Ubenimex occurs as a white crystalline powder.

It is freely soluble in acetic acid (100), slightly soluble in water, and very slightly soluble in ethanol (99.5).

It dissolves in 1 mol/L hydrochloric acid TS.

Melting point: about 230°C (with decomposition).

Identification (1) Determine the absorption spectrum of a

solution of Ubenimex (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_{D}^{20}$: $-15.5 - -17.5^{\circ}$ (after drying, 0.5 g, 1 mol/L hydrochloric acid TS, 50 mL, 100 mm).

Purity (1) Heavy metals $\langle 1.07 \rangle$ —Proceed with 2.0 g of Ubenimex 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 Ubenimex in 10 mL of the mobile phase A, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase A to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly $20 \,\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area of these solutions by the automatic integration method: the area of the peak other than ubenimex obtained from the sample solution is not larger than 1/2 times the peak area of ubenimex from the standard solution. Furthermore, the total area of the peaks other than ubenimex is not larger than the peak area of ubenimex from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 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 μ m in particle diameter).

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

Mobile phase A: A mixture of diluted 0.1 mol/L potassium dihydrogen phosphate TS (13 in 20) and acetonitrile for liquid chromatography (17:3).

Mobile phase B: A mixture of acetonitrile for liquid chromatography and diluted 0.1 mol/L potassium dihydrogen phosphate TS (13 in 20) (2:1).

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

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 - 20	100	0
20 - 60	$100 \rightarrow 0$	$0 \rightarrow 100$
60 - 70	0	100

Flow rate: Adjust the flow rate so that the retention time of ubenimex is about 14 minutes.

Time span of measurement: About 5 times as long as the retention time of ubenimex, beginning after the solvent peak.

System suitability-

Test for required detectability: Pipet 1 mL of the standard

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solution, and add the mobile phase A to make exactly 10 mL. Confirm that the peak area of ubenimex obtained from $20 \,\mu\text{L}$ of this solution is equivalent to 7 to 13% of that from $20 \,\mu\text{L}$ of the standard solution.

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ubenimex are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ubenimex is not more than 2.0%.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (0.5 g, in vacuum, 80°C, 4 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Ubenimex, previously dried, dissolve in 60 mL of acetic acid (100), and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 30.84 mg of $C_{16}H_{24}N_2O_4$

Containers and storage Containers—Tight containers.

Ubenimex Capsules

ウベニメクスカプセル

Ubenimex Capsules contain not less than 93.0% and not more than 107.0% of the labeled amount of ubenimex ($C_{16}H_{24}N_2O_4$: 308.37).

Method of preparation Prepare as directed under Capsules, with Ubenimex.

Identification To a quantity of the contents of Ubenimex Capsules, equivalent to 25 mg of Ubenimex according to the labeled amount, add water to make 50 mL, shake well, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 250 nm and 254 nm, between 255 nm and 259 nm, and between 261 nm and 265 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 capsule of Ubenimex Capsules add 30 mL of a mixture of water and acetonitrile (7:3), shake well for 30 minutes, and add a mixture of water and acetonitrile (7:3) to make exactly 50 mL. Centrifuge this solution and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μ m. Discard the first 5 mL of the filtrate, pipet V mL of the subsequent filtrate, equivalent to about 3 mg of ubenimex (C₁₆H₂₄N₂O₄), add exactly 4 mL of the internal standard solution, add a mixture of water and acetonitrile (7:3) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of ubenimex for assay, previously dried at 80°C for 4 hours under reduced pressure, and dissolve in a mixture of water and acetonitrile (7:3) to make exactly 100 mL. Pipet 15 mL of this solution, add exactly 4 mL of the internal standard solution, add a mixture of water and acetonitrile (7:3) to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of ubenimex to that of the internal standard.

Amount (mg) of ubenimex ($C_{16}H_{24}N_2O_4$) = $M_S \times Q_T/Q_S \times 1/V \times 15/2$

 $M_{\rm S}$: Amount (mg) of ubenimex for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in a mixture of water and acetonitrile (7:3) (1 in 2000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, ubenimex 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 $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ubenimex to that of the internal standard is not more than 2.0%.

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Ubenimex Capsules is not less than 70%.

Start the test with 1 capsule of Ubenimex Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding $0.45 \,\mu\text{m}$. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add a mixture of water and acetonitrile (7:3) to make exactly V'mL so that each mL contains about $11 \mu g$ of ubenimex (C16H24N2O4) according to labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of ubenimex for assay, previously dried in vacume at 80°C for 4 hours, and dissolve in a mixture of water and acetonitrile (7:3) to make exactly 100 mL. Pipet 5 mL of this solution, add a mixture of water and acetonitrile (7:3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of ubenimex in each solution.

Dissolution rate (%) with respect to the labeled amount of ubenimex ($C_{16}H_{24}N_2O_4$)

 $= M_{\rm S} \times A_{\rm T}/A_{\rm S} \times V'/V \times 1/C \times 45$

 $M_{\rm S}$: Amount (mg) of ubenimex for assay

C: Labeled amount (mg) of ubenimex ($C_{16}H_{24}N_2O_4$) in 1 capsule

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 50 μ L of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of Ubenimex are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with $50 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of Ubenimex is not more than 2.0%.

Assay To 10 Ubenimex capsules add 140 mL of a mixture of water and acetonitrile (7:3), shake well for 30 minutes, and add a mixture of water and acetonitrile (7:3) to make exactly 200 mL. Centrifuge this solution, and filter. Discard the first 20 mL of the filtrate, pipet a volume of the subsequent filtrate, equivalent to about 7.5 mg of ubenimex $(C_{16}H_{24}N_2O_4)$, add exactly 10 mL of the internal standard solution, add a mixture of water and acetonitrile (7:3) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of ubenimex for assay, previously dried at 80°C for 4 hours under reduced pressure, dissolve in a mixture of water and acetonitrile (7:3) to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution, and a mixture of water and acetonitrile (7:3) to make 50 mL, and use this solution as the standard solution. Perform the test with $20 \,\mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of ubenimex to that of the internal standard.

Amount (mg) of ubenimex (
$$C_{16}H_{24}N_2O_4$$
)
= $M_S \times Q_T/Q_S \times 1/4$

 $M_{\rm S}$: Amount (mg) of ubenimex for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in mixture of water and acetonitrile (7:3) (1 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 200 nm).

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

Column temperature: A constant temperature of about 30° C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 100) and acetonitrile for liquid chromatography (83:17).

Flow rate: Adjust the flow rate so that the retention time of ubenimex is about 8 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, ubenimex 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 $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ubenimex to that of the internal standard is

not more than 1.0%.

Containers and storage Containers—Tight containers.

Ubidecarenone



$$C_{59}H_{90}O_4$$
: 863.34

(2E,6E,10E,14E,18E,22E,26E,30E,34E,38E)-2-(3,7,11,15,19,23,27,31,35,39-Decamethyltetraconta-2,6,10,14,18,22,26,30,34,38-decaen-1-yl)-5,6-dimethoxy-3-methyl-1,4-benzoquinone [303-98-0]

Ubidecarenone contains not less than 98.0% of $C_{59}H_{90}O_4$, calculated on the anhydrous basis.

Description Ubidecarenone occurs as a yellow to orange crystalline powder. It is odorless and has no taste.

It is soluble in diethyl ether, very slightly soluble in ethanol (99.5), and practically insoluble in water.

It is gradually decomposed and colored by light. Melting point: about 48°C.

Identification (1) Dissolve 0.05 g of Ubidecarenone in 1 mL of diethyl 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 appears.

(2) Determine the infrared absorption spectrum of Ubidecarenone as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Ubidecarenone RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals $\langle 1.07 \rangle$ —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 0.05 g of Ubidecarenone in 50 mL of ethanol (99.5) by warming at about 50°C for 2 minutes, and after cooling use this solution as the sample solution. To exactly 1 mL of the sample solution add ethanol (99.5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> 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 the peak of ubidecarenone from the sample solution is not larger than the peak area of ubidecarenone 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.

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Detection sensitivity: Adjust the detection sensitivity so that the peak height of ubidecarenone obtained from 5 μ L of the standard solution is between 20 mm and 40 mm.

Time span of measurement: About 2 times of the retention time of ubidecarenone beginning after the solvent peak.

Water <2.48> Not more than 0.20% (1 g, volumetric titration, direct titration).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Ubidecarenone and Ubidecarenone RS (separately determined the water $\langle 2.48 \rangle$ in the same manner as Ubidecarenone) dissolve each in 40 mL of ethanol (99.5) by warming at about 50°C for 2 minutes, and after cooling add ethanol (99.5) to make exactly 50 mL each, and use these solutions as the sample solution and standard solution. Perform the test with exact 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and determine peak areas, $A_{\rm T}$ and $A_{\rm S}$, of ubidecarenone of these solutions.

Amount (mg) of
$$C_{59}H_{90}O_4 = M_S \times A_T/A_S$$

 $M_{\rm S}$: Amount (mg) of Ubidecarenone RS, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 275 nm).

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

Column temperature: A constant temperature of about $35^{\circ}C$.

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.

Selection of column: Dissolve 0.01 g each of Ubidecarenone and ubiquinone-9 in 20 mL of ethanol (99.5) by warming at about 50 °C for 2 minutes. After cooling, proceed with $5 \,\mu$ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of ubiquinone-9 and ubidecarenone in this order with the resolution between these peaks being not less than 4.

System repeatability: Repeat the test 5 times with the standard solution under the above operating conditions: the relative standard deviation of the peak areas of ubidecarenone is not more than 0.8%.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Ulinastatin

ウリナスタチン

Ulinastatin is a solution of a glycoprotein having trypsin inhibiting activity, which is separated and purified from human urine.

It contains ulinastatin of not less than 45,000 Units per mL and not less than 2500 Units per mg protein.

Description Ulinastatin occurs as a light brown to brown, clear liquid.

Identification (1) Dilute a suitable amount of Ulinastatin with water to make a solution containing 4000 Units of ulinastatin per mL. To 1 mL of this solution add 1 mL of a solution of phenol (1 in 20), then carefully add 5 mL of sulfuric acid, and mix: an orange to red-orange color develops.

(2) Dilute a suitable quantity of Ulinastatin with water to make a solution containing 2000 units per mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Dilute a suitable amount of Ulinastatin with pH 7.8 2,2',2"-nitrilotrisethanol buffer solution to make a solution containing 500 Units of ulinastatin per mL, and use this solution as the sample solution. Use the same buffer solution as the control solution. To 0.1 mL each of the sample solution and the control solution add 1.6 mL of the buffer solution and 0.2 mL of trypsin TS for test of ulinastatin, mix, and allow them to stand in a water bath at 25°C for 1 minute. Then add 1 mL of N- α -benzoyl-L-arginine-4-nitroanilide TS, mix, and allow them to stand at 25°C for 2 minutes: the solution obtained with the sample solution develops no color while that obtained with the control solution develops a yellow color.

(4) To 1.5 g of Powdered Agar add 100 mL of pH 8.4 boric acid-sodium hydroxide buffer solution, dissolve by warming in a water bath, then pour immediately into a Petri dish placed horizontally so that the agar layer is about 2 mm in thickness. After the agar becomes hard, bore two wells about 2.5 mm in diameter with a separation of 6 mm from each other. In one of the wells place $10 \,\mu$ L of a solution of Ulinastatin containing 500 Units per mL in pH 8.4 boric acid-sodium hydroxide buffer solution, and in the other well place $10 \,\mu$ L of anti-ulinastatin rabbit serum, cover the dish to avoid drying of the agar, and allow to stand for overnight at a room temperature: a clear precipitin line appears between the wells.

pH <2.54> 6.0 - 8.0

Specific activity When calculated from the results obtained by the Assay and the following method, the specific activity is not less than 2500 Units per 1 mg protein.

(i) Sample solution—To an exactly measured volume of Ulinastatin, equivalent to about 10,000 Units according to the labeled amount, add water to make exactly 20 mL.

(ii) Standard solutions—Weigh accurately about 10 mg of bovine serum albumin for test of ulinastatin, and dissolve in water to make exactly 20 mL. To a suitable volume of this solution add water to make four solutions containing exactly 300, 200, 100 and 50 μ g of the albumin per mL, respectively.

(iii) Procedure—Pipet 0.5 mL each of the sample solution and standard solutions, put them in glass test tubes about 18 mm in internal diameter and about 130 mm in length, add exactly 5 mL of alkaline copper TS, mix, and allow the tubes to stand in a water bath at 30°C for 10 minutes. Then add exactly 0.5 mL of diluted Folin's TS (1 in 2), mix, and warm in the water bath for 20 minutes. Determine the absorbances of these solutions at 750 nm as directed under Ultravioletvisible Spectrophotometry $\langle 2.24 \rangle$ using a solution obtained

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in the same manner with 0.5 mL of water as the blank.

Plot the absorbances of the standard solutions on the vertical axis and their protein concentrations on the horizontal axis to prepare a calibration curve, and calculate the protein content of the sample solution from its absorbance by using this curve. Then calculate the amount of protein per mL of Ulinastatin.

Purity (1) Heavy metals $\langle 1.07 \rangle$ —Proceed with 10 mL of Ulinastatin according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 1 ppm).

(2) Related substances—To a suitable volume of Ulinastatin add water to make a solution containing exactly 12,500 Units per mL, and use this solution as the sample stock solution. To exactly 0.25 mL of the sample stock solution add exactly 0.2 mL of glycerin and exactly 0.05 mL of 0.05% bromophenol blue TS, mix, and use this solution as the sample solution. Separately, to exactly 1 mL of the sample stock solution add water to make exactly 100 mL. To exactly 0.25 mL of this solution add exactly 0.2 mL of glycerin and exactly 0.25 mL of this solution add exactly 0.2 mL of glycerin and exactly 0.05 mL of the sample stock solution add water to make exactly 100 mL. To exactly 0.25 mL of this solution add exactly 0.2 mL of glycerin and exactly 0.05 mL of 0.05% bromophenol blue TS, mix, and use this solution as the standard solution. Perform the following test with the sample solution and the standard solution: the bands other than the principal band obtained from the standard solution in the electrophoretogram.

(i) Tris buffer solution for polyacrylamide gel electrophoresis A Dissolve 18.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 80 mL of water, adjust to pH 8.8 with 6 mol/L hydrochloric acid TS, and add water to make 100 mL.

(ii) Tris buffer solution for polyacrylamide gel electrophoresis B Dissolve 6.0 g of 2-amino-2-hydroxymethyl-1,3propanediol in 80 mL of water, adjust to pH 8.8 with 6 mol/L hydrochloric acid TS, and add water to make 100 mL.

(iii) Tris buffer solution for polyacrylamide gel electrophoresis C Dissolve 3.0 g of 2-amino-2-hydroxymethyl-1,3propanediol and 14.4 g of glycine in water to make 1000 mL.

(iv) Acrylamide solution for polyacrylamide gel electrophoresis Dissolve 30 g of acrylamide and 0.8 g of N, N'methylenebisacrylamide in water to make 100 mL.

(v) Gel for separation Mix gently 15 mL of tris buffer solution for polyacrylamide gel electrophoresis A, 20 mL of acrylamide solution for polyacrylamide gel electrophoresis, 24.5 mL of water, 0.022 mL of N, N, N', N'-tetramethyl-ethylenediamine, 0.32 mL of 10% ammonium peroxodisulfate TS and 0.3 mL of 1 mol/L sodium sulfite TS, pour into a plate for slab gel preparation, then cover the gel mixture with a layer of water, and allow to set for 1 hour.

(vi) Gel for concentration Remove the water layer on the gel for separation, and pour a mixture of 2.5 mL of tris buffer solution for polyacrylamide gel electrophoresis B, 2.66 mL of acrylamide solution for polyacrylamide gel electrophoresis, 14.6 mL of water, 0.01 mL of N, N, N', N'-tetramethylethylenediamine, 0.2 mL of 10% ammonium peroxodisulfate TS and 0.04 mL of 1 mol/L sodium sulfite TS on the gel. Then position a plastic sample well former so that the height of the gel for concentration is about 15 mm, and allow to set for 2 hours.

(vii) Procedure

Electrophoresis-Set the gel in an apparatus for slab gel

electrophoresis, and fill the upper and lower reservoirs with tris buffer solution for polyacrylamide gel electrophoresis C. Introduce carefully $10 \,\mu$ L each of the sample solution and standard solution into the wells using a different well for each solution, and allow electrophoresis to proceed using the electrode of the lower reservoir as the anode. Switch off the power supply when the bromophenol blue band has migrated to about 10 mm from the bottom of the gel.

Staining—Dissolve 2.0 g of Coomassie brilliant blue R-250 in a mixture of 400 mL of methanol and 100 mL of acetic acid (100), add water to make 1000 mL, and use this solution as the staining solution. Stain the gel for 2 hours in the staining solution warmed to 40° C.

Decolorization—To 100 mL of methanol and 75 mL of acetic acid (100) add water to make 1000 mL, and use this solution as the rinsing solution. Immerse the gel removed from the staining solution in the rinsing solution to decolorise.

(3) Kallidinogenase—Dilute a suitable volume of Ulinastatin with water so that each mL of the solution contains about 50,000 Units, and use this solution as the sample solution. Take exactly 0.4 mL of the sample solution into a test tube, add exactly 0.5 mL of pH 8.2 tris buffer solution, mix, and allow the tube to stand in a water bath at 37 ± 0.2 °C for 5 minutes. Add exactly 0.1 mL of substrate TS for kallidinogenase assay (4), mix, allow the tube to stand in the water bath of 37 ± 0.2 °C for exactly 30 minutes, then add exactly 0.1 mL of diluted acetic acid (100) (1 in 2), mix, and use this solution as the test solution. Separately, take exactly 0.4 mL of the sample solution in a test tube, add exactly 0.5 mL of pH 8.2 tris buffer solution, mix, and allow the tube to stand in the water bath of 37 ± 0.2 °C for 35 minutes. Then add exactly 0.1 mL of diluted acetic acid (100) (1 in 2), mix, add exactly 0.1 mL of substrate TS for kallidinogenase assay (4), mix, and use this solution as the control solution. Determine the absorbances of the test solution and the control solution at 405 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using water as the blank, and calculate the difference between them: the difference is not more than 0.050.

Molecular mass Dilute a suitable volume of Ulinastatin with the mobile phase so that each mL of the solution contains about 6500 Units, and use this solution as the sample solution. Separately, dissolve 1.0 mg each of γ -globulin (mol. mass: 160,000), bovine serum albumin for test of ulinastatin (mol. mass: 67,000), and myoglobin (mol. mass: 17,000) in about 1 mL of the mobile phase, and use this solution as the molecular mass reference solution. Perform the test with 50 μ L each of the sample solution and molecular mass reference solution as directed under Liquid Chromatography <2.01> according to the following conditions. Prepare a calibration curve by plotting the logarithm of molecular masses on the vertical axis and the retention times (min) of the molecular mass reference substances on the horizontal axis, and determine the molecular mass of the sample using the calibration curve and the retention time obtained with the sample solution: the molecular mass is $67,000 \pm 5000$. Operating conditions—

Detector: An ultraviolet absorption photometer (wave-length: 280 nm).

Column: A stainless steel column about 7 mm in inside diameter and about 60 cm in length, packed with porous silica gel for liquid chromatography (10 – 12 μ m in particle diameter).

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

Mobile phase: Dissolve 16.33 g of potassium dihydrogenphosphate and 124.15 g of ethylene glycol in water to make 1000 mL. If necessary, adjust to pH 4.0 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of bovine serum albumin is about 36 minutes.

Selection of column: Proceed with 50 μ L of the molecular mass reference solution according to the above operating conditions, and calculate the resolution. Use a column from which γ -globulin, bovine serum albumin and myoglobin are eluted in this order with the resolution between their peaks being not less than 1.5, respectively.

Antigenicity Dilute a suitable volume of Ulinastatin with isotonic sodium chloride solution so that each mL of the solution contains 15,000 Units, and use this solution as the sample solution. Inject 0.10 mL of the sample solution on 3 occasions at intervals of 2 days into the peritoneal cavity of each of 4 well-nourished, healthy guinea pigs weighing 250 to 300 g. Inject 0.10 mL of horse serum into the peritoneal cavity of each of 4 guinea pigs of another group as a control. Inject 0.20 mL of the sample solution intravenously into each of 2 guinea pigs of the first group 14 days after the first intraperitoneal injection and into each of the remaining 2 guinea pigs 21 days after the injection, and inject 0.20 mL of horse serum intravenously in the same manner into each guinea pig of the second group. Observe the signs of respiratory distress, collapse or death of the animals for 30 minutes after each intravenous injection and 24 hours later: the animals of the first group exhibit none of the signs mentioned above, and all the animals of the second group exhibit symptoms of respiratory distress or collapse and not less than 3 animals are killed.

Toxicity Inject intravenously 0.50 mL of Ulinastatin into each of five well-fed, healthy albino mice weighing 18 to 25 g: no mouse dies within 48 hours after injection. If any mouse dies within 48 hours, repeat the test using 5 albino mice weighing 19 to 21 g: all the animals survive for 48 hours.

Assay Measure exactly a suitable volume of Ulinastatin, dilute with 2,2',2"-nitrilotrisethanol buffer solution, pH 7.8 so that each mL of the solution contains about 150 Units according to the labeled amount, and use this solution as the sample solution. Separately, dilute a suitable volume of Ulinastatin RS with 2,2',2"-nitrilotrisethanol buffer solution, pH 7.8 so that each mL of the solution contains exactly 300, 200, 100, 50 or 0 Units, and use these solutions as the standard solutions. 2,2',2"-Nitrilotrisethanol buffer solution, pH 7.8 and N-α-benzoyl-L-arginine-4-nitroanilide TS are warmed in a water bath of $25 \pm 1^{\circ}$ C for use as described below. Take exactly 0.1 mL each of the sample solution and the standard solutions in test tubes, add exactly 1.6 mL of 2,2',2"-nitrilotrisethanol buffer solution, pH 7.8 mix, and put the tubes in the water bath of $25 \pm 1^{\circ}$ C. One minute after addition of the buffer solution add exactly 0.2 mL of ice-cooled trypsin TS for test of ulinastatin, mix, and put the tubes again in the water bath. One minute later add exactly 1 mL of $N-\alpha$ -benzoyl-L-arginine-4-nitroanilide TS, mix, and

then put the tubes in the water bath. Exactly 2 minutes later add exactly 0.1 mL of diluted acetic acid (100) (1 in 2) to stop the enzyme reaction, and determine the absorbances of the solutions so obtained at 405 nm as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$ using water as the blank. Prepare a calibration curve using the absorbances obtained with the standard solutions, and calculate ulinastatin Units in the sample solution from its absorbance by using this curve.

Containers and storage Containers—Tight containers. Storage—Not exceeding at -20° C.

Urapidil

ウラピジル



 $\label{eq:C20H29N5O3: 387.48} \\ 6-\{3-[4-(2-Methoxyphenyl)piperazin-1-yl]propylamino\}-1,3-dimethyluracil \\ [34661-75-1] \\ \end{cases}$

Urapidil, when dried, contains not less than 98.0% and not more than 101.0% of $C_{20}H_{29}N_5O_3$.

Description Urapidil occurs as white to pale yellowish, white, crystals or crystalline powder. It has a bitter taste.

It is freely soluble in acetic acid (100), sparingly soluble in ethanol (95) and in acetone, and very slightly soluble in water.

Identification (1) Determine the absorption spectrum of a solution of Urapidil in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Urapidil as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 156 – 161°C

Purity (1) Chloride $\langle 1.03 \rangle$ —Dissolve 3.0 g of Urapidil in 40 mL of acetone and 6 mL of dilute nitric acid, add water to make 50 mL, and 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 40 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.003%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Urapidil 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 40 mg of Urapidil in 5

1552 Urea / Official Monographs

mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) 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 $\langle 2.03 \rangle$. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop with a mixture of ethyl acetate, ethanol (95) and ammonia water (28) (22:13:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot appears not more than one and it is not more intense than the spot from the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.2% (1 g).

Assay Weigh accurately about 70 mg of Urapidil, previously dried, dissolve in 80 mL of acetic acid (100), and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = $12.92 \text{ mg of } C_{20}H_{29}N_5O_3$

Containers and storage Containers—Tight containers.

Urea

尿素

CH₄N₂O: 60.06 Urea [57-13-6]

Urea contains not less than 99.0% of CH_4N_2O .

Description Urea occurs as colorless to white crystals or crystalline powder. It is odorless, and has a cooling, saline taste.

It is very soluble in water, freely soluble in boiling ethanol (95), soluble in ethanol (95), and very slightly soluble in diethyl ether.

A solution of Urea (1 in 100) is neutral.

Identification (1) Heat 0.5 g of Urea: it liquefies and the odor of ammonia is perceptible. Continue heating until the liquid becomes turbid, then cool. Dissolve the resulting lump in a mixture of 10 mL of water and 2 mL of sodium hydroxide TS, and add 1 drop of copper (II) sulfate TS: a reddish purple color develops.

(2) Dissolve 0.1 g of Urea in 1 mL of water, and add 1 mL of nitric acid: a white, crystalline precipitate is formed.

Melting point <2.60> 132.5 - 134.5°C

Purity (1) Chloride $\langle 1.03 \rangle$ —Perform the test with 2.0 g of Urea. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.007%).

(2) Sulfate <1.14>—Perform the test with 2.0 g of Urea. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Urea 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) Ethanol-insoluble substances—Dissolve 5.0 g of Urea in 50 mL of warm ethanol (95), filter through a tared glass filter (G4), wash the residue with 20 mL of warm ethanol (95), and dry at 105° C for 1 hour: the mass of the residue is not more than 2.0 mg.

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Urea, dissolve in water, and make exactly 200 mL. Measure exactly 5 mL of this solution into a Kjeldahl flask, and proceed as directed under Nitrogen Determination $\langle 1.08 \rangle$.

Each mL of 0.005 mol/L sulfuric acid VS = 0.3003 mg of CH₄N₂O

Containers and storage Containers-Well-closed containers.

Urokinase

ウロキナーゼ

[9010-53-1]

Urokinase is an enzyme, obtained from human urine, that activates plasminogen, and has the molecular mass of about 54,000.

It is a solution using a suitable buffer solution as the solvent.

It contains not less than 60,000 Units per mL, and not less than 120,000 Units per mg of protein.

Description Urokinase is a clear and colorless liquid. The pH is between 5.5 and 7.5.

Identification (1) Dissolve 0.07 g of fibrinogen in 10 mL of phosphate buffer solution, pH 7.4. To this solution add 1 mL of a solution of thrombin containing 10 Units per mL in isotonic sodium chloride solution, mix, place in a Petri dish about 90 mm in inside diameter, and keep horizontally until the solution is coagulated. On the surface drop $10 \,\mu$ L of a solution of Urokinase containing 100 Units per mL in gelatin-tris buffer solution, and stand for 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 come 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 $10 \,\mu$ L of a solution of Urokinase containing 30,000 Units per mL in isotonic sodium chloride solution and $10 \,\mu$ L of anti-urokinase serum, and stand for overnight: a clear precipitin line is appeared.

Purity (1) Heavy metals $\langle 1.07 \rangle$ —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 iso-

tonic sodium chloride solution so that each mL of the solution contains 12,000 Units, and use this solution as the sample solution. To anti-A type antibody for blood typing add isotonic sodium chloride solution to dilute each 32, 64, 128, 256, 512 and 1024 times, place separately $25 \,\mu$ L each of these solutions in six wells on the first and second lane of a V-shaped 96-wells microplate. Next, add $25 \,\mu$ L of the sample solution into the six wells on the first lane and $25 \,\mu$ L of isotonic sodium chloride solution into the six wells of the second lane, mix, and allow to stand for 30 min. To each well add $50 \,\mu$ L 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 by using anti-B type antibody for blood typing and B-type erythrocyte suspension.

Abnormal toxicity Dilute Urokinase with isotonic sodium chloride solution so that each mL of the solution contains 12,000 Units, and use this solution as the sample solution. Inject 5.0 mL of the sample solution into the peritoneal cavity of each of 2 or more of well-nourished, healthy guinea pigs weighing about 350 g. 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 10,000 Units, and use this solution as the sample solution. Perform the test with 100 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak 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 inside diameter and 60 cm in length, packed with porous silica gel for liquid chromatography (10 to $12 \,\mu$ m in particle diameter).

Column temperature: A constant temperature of about 20° C.

Reaction coil: A stainless steel column 0.25 mm in inside 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 per minute.

Reaction reagent: 7-(Glutarylglycyl-L-arginylamino)-4-methylcoumarin TS.

Flow rate of reaction reagent: 0.75 mL per 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 20,000 Units per mL. Proceed with $100 \,\mu$ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of high molecular mass urokinase (mol. wt.: 54,000) and low molecular mass urokinase (mol. wt.: 33,000) in this order with the resolution between these peaks being not less than 1.0.

Assay (1) Urokinase-Pipet 1 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 sample solution. Add exactly 2 mL of gelatin-tris buffer solution to contents of one ampoule of High-Molecular Mass Urokinase RS to dissolve, pipet 1 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-pyroglutamylglycyl-L-arginine-p-nitroaniline hydrochloride TS in two silicon-coated test tubes about 10 mm in inside diameter. warm them in a water bath at 35 ± 0.2 °C for 5 minutes, add separately 0.50 mL each of the sample solution and the standard solution, warm in a water bath at 35 ± 0.2 °C for exactly 30 minutes, then add 0.50 mL of diluted acetic acid (100) (2 in 5). Determine the absorbances, $A_{\rm T}$ and $A_{\rm S}$, of these solutions at 405 nm as directed under Ultravioletvisible Spectrophotometry $\langle 2.24 \rangle$, using water as the blank. Separately place 1.0 mL of L-pyroglutamylglycyl-L-argininep-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 sample 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.

> Amount (Units) of Urokinase = $(A_{\rm T} - A_{\rm T0})/(A_{\rm S} - A_{\rm S0}) \times a \times b$

a: Amount (Units) of urokinase in 1 mL of the standard solution

b: Total volume (mL) of the sample solution

(2) Protein—Measure exactly a volume of Urokinase, equivalent to about 15 mg of protein, and perform the test as directed under Nitrogen Determination $\langle 1.08 \rangle$.

Each mL of 0.005 mol/L sulfuric acid VS = 0.8754 mg of protein

Containers and storage Containers—Tight containers. Storage—Not exceeding -20° C.

Ursodeoxycholic Acid

Ursodesoxycholic Acid

ウルソデオキシコール酸



 $C_{24}H_{40}O_4$: 392.57 3 α ,7 β -Dihydroxy-5 β -cholan-24-oic acid [128-13-2]

Ursodeoxycholic Acid, when dried, contains not less than 98.5% and not more than 101.0% of $C_{24}H_{40}O_4$.

Description Ursodeoxycholic Acid occurs as a white crystal or powder, with bitter taste.

It is freely soluble in methanol, in ethanol (99.5) and in acetic acid (100), and practically insoluble in water.

Identification Determine the infrared absorption spectrum of Ursodeoxycholic Acid as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $\langle 2.49 \rangle$ [α]_D²⁰: +59.0 - +62.0° (after drying, 1.0 g, ethanol (99.5), 25 mL, 100 mm).

Melting point <2.60> 200 – 204°C

Purity (1) Sulfate $\langle 1.14 \rangle$ —Dissolve 2.0 g of Ursodeoxycholic Acid in 20 mL of acetic acid (100), add water to make 200 mL, 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 sample solution. To 40 mL of the sample solution 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 by adding 4 mL of acetic acid (100), 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(2) Heavy metal $\langle 1.07 \rangle$ —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).

(3) Barium—To 2.0 g of Ursodeoxychollic Acid add 100 mL of water and 2 mL of hydrochloric acid, boil for 2 minutes, allow it to cool, filter, and wash the filter with water until to get 100 mL of the filtrate. To 10 mL of the filtrate add 1 mL of dilute sulfuric acid: no turbidity is appeared.

(4) Related substances—Dissolve 0.10 g of Ursodeoxycholic Acid in 1 mL of methanol, add acetone to make exactly 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetone to make exactly 100 mL. Pipet 1 mL and 2 mL of this solution, to each add acetone to make exactly 20 mL, and use these solutions as the standard solution (A) and the standard solution (B), respectively. Separately, dissolve 50 mg of chenodeoxycholic acid for thin-layer chromatography in 5 mL of methanol, add acetone to make exactly 50 mL. Pipet 2 mL of this solution, add acetone to make exactly 20 mL, and use this solution as the standard solution (1). Furthermore, dissolve 25 mg of lithocholic acid for thin-layer chromatography in 5 mL of methanol, and add acetone to make exactly 50 mL. Pipet 2 mL of this solution, and add acetone to make exactly 20 mL. Pipet 2 mL of this solution, add acetone to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 10 μ L each of the sample solution, standard solutions (1), (2), (A) and (B) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of isooctane, ethanol (99.5), ethyl acetate and acetic acid (100) (10:6:3:1) to a distance of about 15 cm, and air-dry the plate. Dry the plate further at 120°C for 30 minutes, and immediately spray evenly the solution which was prepared by dissolving 5 g of phosphomolybdic acid n-hydrate in about 50 mL of ethanol (99.5), to which 5 mL of sulfuric acid is dropped in and add ethanol (99.5) to make 100 mL, and heat at 120°C for 3 to 5 minutes: the spots from the sample solution corresponding to the spots obtained from the standard solution (1) and (2) are not more intense than the spots from the standard solutions (1) and (2), the spots other than the principal spot and other than those mentioned above from the sample solution are not intense than the spots obtained from the standard solution (B), and the total amount of the spots other than the principal spot and other than those mentioned above from the sample solution, which is calculated by the comparison with the spots obtained from the standard solutions (A) and (B), is not more than 0.25%.

Loss on drying $\langle 2.41 \rangle$ Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.2% (1 g).

Assay Weigh accurately about 0.5 g of Ursodeoxycholic Acid, previously dried, dissolve in 40 mL of ethanol (95) and 20 mL of water, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS = $39.26 \text{ mg of } C_{24}H_{40}O_4$

Containers and storage Containers-Well-closed containers.

Ursodeoxycholic Acid Granules

ウルソデオキシコール酸顆粒

Ursodeoxycholic Acid Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of ursodeoxycholic acid ($C_{24}H_{40}O_4$: 392.57).

Method of preparation Prepare as directed under Granules, with Ursodeoxycholic Acid.

Identification To a quantity of powdered Ursodeoxycholic

Acid Granules, equivalent to 20 mg of Ursodeoxycholic Acid according to the labeled amount, add 10 mL of methanol, and shake for 20 minutes. Centrifuge this solution, pipet 4 mL of the supernatant liquid, and evaporate the methanol under reduced pressure. To the residue add 4 mL of acetone, disperse with ultrasonic waves, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of ursodeoxycholic acid in 5 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 10 μ L of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of isooctane, ethanol (99.5), ethyl acetate and acetic acid (100) (10:6:3:1) to a distance of about 15 cm, and air-dry the plate. Dry the plate further at 120°C for 30 minutes, immediately splay evenly a solution of phosphomolybdic acid n-hydrate in ethanol (99.5) (1 in 5), and heat at 120°C for 3 to 5 minutes: the principle spot obtained from the sample solution and the spot from the standard solution show a blue color and the same Rf value.

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 15 minutes of Ursodeoxycholic Acid Granules is not less than 80%.

Start the test with an accurately weigh amount of Ursodeoxycholic Acid Granules, equivalent to about 50 mg of ursodeoxycholic acid $(C_{24}H_{40}O_4)$ according to labeled amount, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding $0.45 \,\mu\text{m}$. Discard the first 10 mL of the filtrate, use the subsequent filtrate as the sample solution. Separately, weigh accurately about 22 mg of ursodeoxycholic acid for assay, previously dried at 105°C for 2 hours, and dissolve in acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly $100 \,\mu\text{L}$ each of sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of ursodeoxycholic acid of each solution.

Dissolution rate (%) with respect to the labeled amount of ursodeoxycholic acid $(C_{24}H_{40}O_4)$

 $= M_{\rm S}/M_{\rm T} \times A_{\rm T}/A_{\rm S} \times 1/C \times 225$

 $M_{\rm S}$: Amount (mg) of ursodeoxycholic acid for assay $M_{\rm T}$: Amount (g) of Ursodeoxycholic Acid Glanules C: Labeled amount (mg) of ursodeoxycholic acid $(C_{24}H_{40}O_4)$ in 1 g

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 100 μ L of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of ursodeoxycholic acid are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with $100 \,\mu\text{L}$ of the standard solution under the above operat-

ing conditions, the relative standard deviation of the peak area of ursodeoxycholic acid is not more than 2.0%.

Assay Weigh accurately an amount of powdered Ursodeoxycholic Acid Granules, equivalent to about 0.1 g of ursodeoxycholic acid ($C_{24}H_{40}O_4$), add exactly 20 mL of the internal standard solution, shake for 10 minutes, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μ m, and use the filtrate as the sample solution. Separately, weigh accurately about 0.1 g of ursodeoxycholic acid for assay, previously dried at 105°C for 2 hours, dissolve in exactly 20 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of ursodeoxycholic acid to that of the internal standard.

Amount (mg) of ursodeoxycholic acid (C₂₄H₄₀O₄) = $M_{\rm S} \times Q_{\rm T}/Q_{\rm S}$

 $M_{\rm S}$: Amount (mg) of ursodeoxycholic acid for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in diluted methanol (4 in 5) (7 in 200,000). *Operating conditions*—

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

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

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

Mobile phase: A mixture of diluted phosphoric acid (1 in 500) and acetonitrile for liquid chromatography (11:9).

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

System suitability-

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, ursodeoxycholic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ursodeoxycholic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Ursodeoxycholic Acid Tablets

ウルソデオキシコール酸錠

Ursodeoxycholic Acid Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of Ursodeoxycholic Acid ($C_{24}H_{40}O_4$: 392.57).

Method of preparation Prepare as directed under Tablets, with Ursodeoxycholic Acid.

Identification To a quantity of powdered Ursodeoxycholic Acid Tablets, equivalent to 20 mg of Ursodeoxycholic Acid

according to the labeled amount, add 10 mL of methanol, and shake for 20 minutes. Centrifuge this solution, pipet 4 mL of the supernatant liquid, and evaporate the methanol under reduced pressure. To the residue add 4 mL of acetone, disperse with ultrasonic waves, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of ursodeoxycholic acid in 5 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of isooctane, ethanol (99.5), ethyl acetate and acetic acid (100) (10:6:3:1) to a distance of about 15 cm, and air-dry the plate. Dry the plate further at 120°C for 30 minutes, and immediately splay evenly a solution of phosphomolybdic acid n-hydrate in ethanol (95) (1 in 5) on the plate, and heat at 120°C for 3 to 5 minutes: the principal spot obtained from the sample solution and the spot from the standard solution show a blue color and the same Rf value.

Uniformity of dosage units $\langle 6.02 \rangle$ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take 1 tablet of Ursodeoxycholic Acid Tablets and add exactly V mL of the internal standard solution so that each mL contains about 5 mg of ursodeoxycholic acid ($C_{24}H_{40}O_4$), disperse it with ultrasonic waves, then agitate to mix for 10 minutes and then centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μ m, and use the filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of ursodeoxycholic acid ($C_{24}H_{40}O_4$) = $M_S \times Q_T/Q_S \times V/20$

 $M_{\rm S}$: Amount (mg) of ursodeoxycholic acid for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in diluted methanol (4 in 5) (7 in 200,000).

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rates in 30 minutes of a 50-mg tablet and in 45 minutes of a 100-mg tablet are not less than 80% and not less than 70%, respectively.

Start the test with 1 tablet of Ursodeoxycholic Acid Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μ m. Discard the first 10 mL of the filtrate and pipet V mL of the subsequent filtrate. Add the dissolution medium to make exactly V'mL so that each mL contains about 56 μ g of ursodeoxycholic acid $(C_{24}H_{40}O_4)$ according to the labeled amount, and use the solution as the sample solution. Separately weigh accurately about 22 mg of ursodeoxycholic acid for assay, previously dried at 105°C for 2 hours, and dissolve in acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly $100 \,\mu\text{L}$ each of sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of ursodeoxycholic acid in each solution.

Dissolution rate (%) with respect to the labeled amount of ursodeoxycholic acid ($C_{24}H_{40}O_4$)

 $= M_{\rm S} \times A_{\rm T}/A_{\rm S} \times V'/V \times 1/C \times 225$

 $M_{\rm S}$: Amount (mg) of ursodeoxycholic acid for assay

C: Labeled amount (mg) of ursodeoxycholic acid in 1 tablet $(C_{24}H_{40}O_4)$

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 100 μ L of the standard solution under the above operating condition, the number of theoretical plates and symmetry factor of the peak of ursodeoxycholic acid are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with $100 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ursodeoxycholic acid is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Ursodeoxycholic Acid Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of ursodeoxycholic acid ($C_{24}H_{40}O_4$), add exactly 20 mL of the internal standard solution, shake for 10 minutes, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μ m, and use the filtrate as the sample solution. Separately, weigh accurately about 0.1 g of ursodeoxycholic acid for assay, previously dried at 105°C for 2 hours, dissolve in exactly 20 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with $10 \,\mu L$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of ursodeoxycholic acid to that of the internal standard.

Amount (mg) of ursodeoxycholic acid (
$$C_{24}H_{40}O_4$$
)
= $M_S \times Q_T/Q_S$

 $M_{\rm S}$: Amount (mg) of ursodeoxycholic acid for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in diluted methanol (4 in 5) (7 in 200,000). *Operating conditions*—

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

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

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

Mobile phase: A mixture of diluted phosphoric acid (1 in 500) and acetonitrile for liquid chromatography (11:9).

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

System suitability-

System performance: When the procedure is run with 10 μ L of the standard solution according to the above operating conditions, ursodeoxycholic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

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System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ursodeoxycholic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

L-Valine

L-バリン

C₅H₁₁NO₂: 117.15 (2*S*)-2-Amino-3-methylbutanoic acid [72-18-4]

L-Valine, when dried, contains not less than 98.5% of $C_5H_{11}NO_2$.

Description L-Valine occurs as white crystals or crystalline powder. It is odorless or has a faint characteristic odor, and has a slightly sweet taste, which becomes bitter.

It is freely soluble in formic acid, soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

Identification Determine the infrared absorption spectrum of L-Valine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_D^{20}$: $+26.5 - +29.0^\circ$ (after drying, 2 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH $\langle 2.54 \rangle$ 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 $\langle 1.03 \rangle$ —Perform the test 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 $\langle 1.14 \rangle$ —Perform the test 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 $\langle 1.02 \rangle$ —Perform the test 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 $\langle 1.07 \rangle$ —Proceed with 1.0 g of L-Valine 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) Arsenic $\langle 1.11 \rangle$ —Proceed with 1.0 g of L-Valine, prepare the test solution 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 sample solution.Pipet 1 mL of the sample 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 these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 5 μ L each of the sample solution and 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 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 sample solution are not more intense than the spot from the standard solution.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = $11.72 \text{ mg of } C_5 H_{11} NO_2$

Containers and storage Containers—Tight containers.

Vancomycin Hydrochloride





C₆₆H₇₅Cl₂N₉O₂₄.HCl: 1485.71

(1S,2R,18R,19R,22S,25R,28R,40S)-50-[3-Amino-2,3,6-trideoxy-3-*C*-methyl- α -L-*lyxo*-hexopyranosyl- $(1\rightarrow 2)$ - 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-21,24,27,41,43pentaazaoctacyclo[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),34,36,38,45,47pentadecaene-40-carboxylic acid monohydrochloride [*1404-93-9*]

Vancomycin Hydrochloride is the hydrochloride of a glycopeptide substance having antibacterial activity produced by the growth of *Streptomyces orientalis*.

It contains not less than $1000 \,\mu g$ (potency) and not more than $1200 \,\mu g$ (potency) per mg, calculated on the anhydrous basis. The potency of Vancomycin Hydrochloride is expressed as mass (potency) of vancomycin (C₆₆H₇₅Cl₂N₉O₂₄: 1449.25).

Description Vancomycin Hydrochloride occurs as a white powder.

It is freely soluble in water, soluble in formamide, slightly soluble in methanol, very slightly soluble in ethanol (95), and practically insoluble in acetonitrile.

It is hygroscopic.

Identification (1) Determine the absorption spectrum of a solution of Vancomycin Hydrochloride (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Vancomycin Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Vancomycin Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Vancomycin Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 20 mg of Vancomycin Hydrochloride in 10 mL of water, and add 1 drop of silver nitrate TS: a white turbidity is produced.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_{D}^{20}$: $-30 - -40^{\circ}$ (0.2 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH $\langle 2.54 \rangle$ 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 $\langle 1.07 \rangle$ —Proceed with 1.0 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 0.10 g of Vancomycin Hydrochloride in 10 mL of the mobile phase A, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase A to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under the Liquid Chromatography <2.01> according to the following conditions. If necessary, proceed with $20 \,\mu\text{L}$ of the mobile phase A in the same manner to compensate for the base line. Determine each peak area by the automatic integration method: the area of each peak other than vancomycin from the sample solution is not larger than the peak area of vancomycin from the standard solution, and the total area of the peaks other than vancomycin is not larger than 3 times of the peak area of vancomycin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 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 μ m in particle diameter).

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

Mobile phase A: A mixture of triethylamine buffer solution, pH 3.2, acetonitrile and tetrahydrofuran (92:7:1). Adjust the amount of acetonitrile so that the retention time of vancomycin is 7.5 to 10.5 minutes.

Mobile phase B: A mixture of triethylamine buffer solution, pH 3.2, acetonitrile and tetrahydrofuran (70:29:1).

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

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 - 12	100	0
12 - 20	$100 \rightarrow 0$	$0 \rightarrow 100$
20 - 22	0	100

Flow rate: 1.5 mL per minute.

Time span of measurement: As long as about 2.5 times of the retention time of vancomycin beginning after the solvent peak.

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System suitability-

Test for required detectability: Confirm that the peak area of vancomycin obtained from $20 \,\mu\text{L}$ of the standard solution is equivalent to 3 to 5% of that from $20 \,\mu\text{L}$ of the sample solution.

System performance: Dissolve 5 mg of Vancomycin Hydrochloride in 10 mL of water, heat at 65°C for 48 hours, and cool to the ordinal temperature. When the procedure is run with 20 μ L of this solution under the above operating conditions, related substance 1, vancomycin and related substance 2 are eluted in this order, the resolution between the peaks of the related substance 1 and vancomycin is not less than 3, the number of theoretical plates of the peak of vancomycin is not less than 1500, and the related substance 2 is eluted between 15 and 18 minutes.

System repeatability: When the test is repeated 5 times with $20 \,\mu\text{L}$ 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%.

Water $\langle 2.48 \rangle$ 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 $\langle 2.44 \rangle$ Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics $\langle 4.02 \rangle$ according to the following conditions.

(i) Test organism-Bacillus subtilis ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 6.2 to 6.4 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Vancomycin Hydrochloride RS, equivalent to about 25 mg (potency), dissolve in water to make exactly 25 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below, and use within 7 days. Take exactly a suitable amount of the standard stock solution, pH 4.5 to make solutions so that each mL contains $100 \,\mu g$ (potency) and $25 \,\mu g$ (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Vancomycin Hydrochloride, equivalent to about 25 mg (potency), and dissolve in water to make exactly 25 mL. Take exactly 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 μ g (potency) and 25 μ g (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers-Tight containers.

Vancomycin Hydrochloride for Injection

注射用バンコマイシン塩酸塩

Vancomycin Hydrochloride for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 115.0% of the labeled amount of vancomycin (C₆₆H₇₅Cl₂N₉O₂₄: 1449.25).

Method of preparation Prepare as directed under Injections, with Vancomycin Hydrochloride.

Description Vancomycin Hydrochloride for Injection occurs as white masses or a white powder.

Identification (1) Dissolve an amount of Vancomycin Hydrochloride for Injection, equivalent to 5 mg (potency) of Vancomycin Hydrochloride, in 50 mL of water, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: it exhibits a maximum between 279 and 283 nm.

(2) Dissolve an amount of Vancomycin Hydrochloride 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 $\langle 2.54 \rangle$ 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 to pale yellow, and the absorbance of the solution, determined at 465 nm as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, is not more than 0.05.

(2) 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 A, and use this solution as the sample solution.

Proceed as directed in the Purity (2) under Vancomycin Hydrochloride.

Water <2.48> Not more than 5.0% (0.1 g, volumetric titration, direct titration. Use a mixture of formamide for Karl Fisher method and methanol for Karl Fisher method (3:1)).

Bacterial endotoxins <4.01> Less than 0.25 EU/mg (potency).

Uniformity of dosage unit <6.02> It meets the requirement of the Mass variation test.

Foreign insoluble matter $\langle 6.06 \rangle$ Perform the test according to the Method 2: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism, culture medium, and standard solutions—Proceed as directed in the Assay under Vancomycin Hydrochloride.

(ii) Sample solutions—Weigh accurately the contents of not less than 10 Vancomycin Hydrochloride for Injection. Weigh accurately an amount of the content, equivalent to about 25 mg (potency) of Vancomycin Hydrochloride according to the labeled amount, and dissolve in water to make exactly 25 mL. Take exactly 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 μ g (potency) and 25 μ g (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Hermetic containers.

Vasopressin Injection

バソプレシン注射液

Vasopressin Injection is an aqueous solution for injection.

It 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.

It contains not less than 85% and not more than 120% 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. It 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.

(i) 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.

(ii) Standard solution: Dilute the standard stock solution with isotonic sodium chloride solution so that each mL of the solution contains 0.020 oxytocin Units.

(iii) Sample solution: Assume oxytocin Units as equivalent to 6/100 of the determined vasopressin Units. Dilute Vasopressin Injection with isotonic sodium chloride solution so that each mL of the resulting solution is expected to contain 0.020 oxytocin Unit.

(iv) 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 suspending the uterus.

(v) 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.

(vi) 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 the chamber, and connect one horn of the uterus to the lever with a thread. If necessary, weigh the lever provided that the mass 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 sample 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 sample 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.

Bacterial endotoxins <4.01> Less than 15 EU/vasopressin Unit.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter $\langle 6.06 \rangle$ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay (i) Test animals: Use healthy male rats weighing between 200 g and 300 g.

(ii) Standard stock solution: Dissolve 2000 Units of Vasopressin RS, according to the labeled Units, in exactly 100 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.

(iii) Standard solution: Dilute the standard stock solution with isotonic sodium chloride solution 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 (vi), and designate this solution as the high-dose standard solution (S_H). Then dilute this solution with isotonic sodium chloride solution 1.5 to 2.0 times by volume, and designate it as the low-dose standard solution (S_L).

(iv) Sample solution: Dilute an accurately measured volume of Vasopressin Injection with isotonic sodium chloride solution 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 sample solution (T_H). Then dilute this solution with isotonic sodium chloride solution 1.5 to 2.0 times by volume, and

designate it as the low-dose sample 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.

(v) 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.

(vi) Procedure: Inject subcutaneously 0.7 mL of a solution of ethyl carbamate (1 in 4) per 100 g of body mass 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 a cannula filled with isotonic sodium chloride solution into a 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 solution, and then immediately inject 0.3 mL of isotonic sodium chloride solution. Insert a cannula into a carotid artery, and connect the cannula to a manometer for blood pressure measurement with a vinyl tube. The cannula and the vinyl tube have previously been filled with isotonic sodium chloride solution. Inject the standard and the sample solutions at regular intervals of 10 to 15 minutes into the femoral vein through the cannula followed by 0.3 mL of the isotonic solution when the blood pressure increases caused by each solution returns to the original level. Measure the height of blood pressure increases within 1 mmHg on the kymogram. Maintain a constant temperature between 20°C and 25°C during the assay. In advance, make four pairs from $S_{\rm H},\,S_{\rm L},\,T_{\rm H},\,T_{\rm L}$ 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.

(vii) Calculation: Subtract increases of blood pressure caused by the low dose from those caused by the high dose in the Pair 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 , for each set to obtain Y_1 , and obtain Y_2 , Y_3 and Y_4 in the same way.

Units in each mL of Vasopressin Injection

= antilog
$$M \times (\text{Units in each mL of the S}_{\text{H}}) \times b/a$$

 $M = (IY_a/Y_b)$
 $I = \log (S_{\text{H}}/S_L) = \log (T_{\text{H}}/T_L)$
 $Y_a = -Y_1 + Y_2 + Y_3 - Y_4$
 $Y_b = Y_1 + Y_2 + Y_3 + Y_4$

a: Volume (mL) of Vasopressin Injection sampled.

b: Total volume (mL) of the high-dose sample solution prepared by diluting with isotonic sodium chloride solution.

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.

$$L = 2\sqrt{(C-1)(CM^2 + I^2)}$$

$$C = \{Y_{\rm b}^2 / (Y_{\rm b}^2 - 4fs^2t^2)\}$$

f: Number of sets

$$s^{2} = \{ \sum y^{2} - (Y/f) - (Y'/4) + (Y_{b}^{2}/4f) \} / r$$

 Σy^2 : The sum of the squares of y_1 , y_2 , y_3 and y_4 .

$$Y = Y_1^2 + Y_2^2 + Y_3^2 + Y_4^2$$

Y': The sum of the squares of the sum of y_1 , y_2 , y_3 and y_4 in each set.

$$n = 3(f - 1)$$

 t^2 : Value shown in the following table against *n* for which s^2 is calculated.

n	$t^2 = F_1$	n	$t^2 = F_1$	n	$t^2 = F_1$
1	161.45	13	4.667	25	4.242
2	18.51	14	4.600	26	4.225
3	10.129	15	4.543	27	4.210
4	7.709	16	4.494	28	4.196
5	6.608	17	4.451	29	4.183
6	5.987	18	4.414	30	4.171
7	5.591	19	4.381	40	4.085
8	5.318	20	4.351	60	4.001
9	5.117	21	4.325	120	3.920
10	4.965	22	4.301	∞	3.841
11	4.844	23	4.279		
12	4.747	24	4.260		

Containers and storage Containers—Hermetic containers. Storage—In a cold place, and avoid freezing.

Expiration date 36 months after preparation.

Verapamil Hydrochloride

Iproveratril Hydrochloride

ベラパミル塩酸塩



 $C_{27}H_{38}N_2O_4.HCl: 491.06 \\ (2RS)-5-[(3,4-Dimethoxyphenethyl)methylamino]-2-(3,4-dimethoxyphenyl)-2-(1-methylethyl)pentanenitrile monohydrochloride$

[152-11-4]

Verapamil Hydrochloride, when dried, contains not less than 98.5% of $C_{27}H_{38}N_2O_4$.HCl.

Description Verapamil Hydrochloride occurs as a white, crystalline powder. It is odorless.

It is freely soluble in methanol, in acetic acid (100) and in chloroform, soluble in ethanol (95) and in acetic anhydride, sparingly soluble in water, and practically insoluble in diethyl ether.

Identification (1) To 2 mL of a solution of Verapamil Hydrochloride (1 in 50) add 5 drops of Reinecke salt TS: a light red precipitate is produced.

(2) Determine the absorption spectrum of a solution of Verapamil Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Verapamil Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Verapamil Hydrochloride (1 in 50) responds to the Qualitative Tests $\langle 1.09 \rangle$ for chloride.

Melting point <2.60> 141 – 145°C.

pH $\langle 2.54 \rangle$ 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 $\langle 1.07 \rangle$ —Proceed with 1.0 g of Verapamil 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) Arsenic $\langle 1.11 \rangle$ —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 exactly 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 100 mL, and use this solution as the standard stock solution. Pipet 5 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 mL of standard stock solution, add chloroform to make exactly 50 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot $10 \,\mu\text{L}$ each of the sample solution and 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 diethylamine (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 other than the principal spot and the original point from the sample solution, are not more intense than the spot from the standard solution (2) in color. The remaining spots from the sample 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 $\langle 2.41 \rangle$ Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

> Each mL of 0.1 mol/L perchloric acid VS = $49.11 \text{ mg of } C_{27}H_{38}N_2O_4.HCl$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Verapamil Hydrochloride Tablets

ベラパミル塩酸塩錠

Verapamil Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of verapamil hydrochloride ($C_{27}H_{38}N_2O_4$.HCl: 491.06).

Method of preparation Prepare as directed under Tablets, with Verapamil Hydrochloride.

Identification (1) To a quantity of pulverized Verapamil Hydrochloride Tablets, equivalent to 0.2 g of Verapamil Hydrochloride according to the labeled amount, add 70 mL of 0.02 mol/L hydrochloric acid TS, and shake occasionally in a water bath at 60°C. After cooling, add 0.02 mol/L hydrochloric acid TS to make 100 mL, and filter. To 3 mL of the filtrate add several drops of Reinecke's salt TS: a light red precipitate is formed.

(2) To 2 mL of the filtrate obtained in (1) add 0.02 mol/L hydrochloric acid TS to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: it exhibits maxima between 227 nm and 231 nm, and between 276 nm and 280 nm.

Uniformity of dosage unit <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Verapamil Hydrochloride Tablets add 70 mL of 0.02 mol/L hydrochloric acid TS, disintegrate the tablet by occasional shaking in a water bath at 60°C for about 30 minutes, and then shake for 5 minutes. After cooling, add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, take exactly *V* mL of the subsequent filtrate, add 0.02 mol/L hydrochloric acid TS to that each mL contains about $40 \,\mu g$ of verapamil hydrochloride (C₂₇H₃₈N₂O₄.HCl), and use this solution as the sample solution. Hereafter, proceed as directed in the Assay.

Amount (mg) of verapamil hydrochloride ($C_{27}H_{38}N_2O_4$.HCl) = $M_S \times A_T/A_S \times V'/V \times 1/25$

 $M_{\rm S}$: Amount (mg) of verapamil hydrochloride for assay

Assay To 10 tablets of Verapamil Hydrochloride Tablets add 140 mL of 0.02 mol/L hydrochloric acid TS, disintegrate the tablets by occasional shaking in a water bath at 60°C for about 30 minutes, and then shake for 5 minutes. After cooling, add 0.02 mol/L hydrochloric acid TS to make exactly 200 mL, and filter. Discard the first 20 mL of the filtrate, take an exact volume of the subsequent filtrate, equivalent to about 4 mg of verapamil hydrochloride (C_{27}H_{38}N_2O_4.HCl), add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of verapamil hydrochloride for assay, previously dried at 105°C for 2 hours, and dissolve in 70 mL of 0.02 mol/L hydrochloric acid TS by occasional shaking in a water bath at 60°C. After cooling, add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 4 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, $A_{\rm T}$ and $A_{\rm S}$, of the sample solution and standard solution at 278 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of verapamil hydrochloride ($C_{27}H_{38}N_2O_4$.HCl) = $M_S \times A_T/A_S \times 1/25$

 $M_{\rm S}$: Amount (mg) of verapamil hydrochloride for assay

Containers and storage Containers—Tight containers.

Vinblastine Sulfate

ビンブラスチン硫酸塩



C₄₆H₅₈N₄O₉.H₂SO₄: 909.05

Methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-acetoxy-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-3azacycloundecino[5,4-*b*]indol-9-yl]-5-hydroxy-8methoxy-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 $C_{46}H_{58}N_4O_9.H_2SO_4$, calculated on the dried basis.

Description Vinblastine Sulfate occurs as a white to pale yellow powder.

It is soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (99.5).

It is hygroscopic.

Optical rotation $[\alpha]_D^{20}$: $-28 - -35^\circ$ (0.20 g calculated on the dried basis, methanol, 10 mL, 100 mm).

Identification (1) Determine the absorption spectrum of a solution of Vinblastine Sulfate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum or the

spectrum of a solution of Vinblastine Sulfate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Vinblastine Sulfate as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Vinblastine Sulfate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Vinblastine Sulfate (1 in 100) responds to the Qualitative Tests $\langle 1.09 \rangle$ for sulfate.

pH $\langle 2.54 \rangle$ 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 50 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 sample solution. Pipet 1 mL of the sample solution, add water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 200 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area of these solutions by the automatic integration method: the area of peak other than the main peak obtained from sample solution is not larger than 1/4 times the peak area of the peaks other than the main peak is not larger than 3/4 times 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.

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

System suitability—

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 μ L of this solution is equivalent to 1.7 to 3.3% of that from 200 μ L of the standard solution.

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

System repeatability: When the test is repeated 6 times with $200 \,\mu\text{L}$ 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%.

Loss on drying Perform the test with about 10 mg of Vinblastine Sulfate as directed in Method 2 under the Thermal Analysis $\langle 2.52 \rangle$ according to the following conditions: not more than 15.0%.

Operating conditions—

Heating rate: 5°C per minute.

Temperature range: room temperature to 200°C.

Atmospheric gas: dried Nitrogen.

Flow rate of atmospheric gas: 40 mL per minute.

Assay Weigh accurately about 10 mg each of Vinblastine Sulfate and Vinblastine Sulfate RS (previously determine the loss on drying in the same conditions as Vinblastine Sulfate),

dissolve in water to make exactly 25 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly $20 \,\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of vinblastine.

Amount (mg) of $C_{46}H_{58}N_4O_9$. $H_2SO_4 = M_S \times A_T/A_S$

 $M_{\rm S}$: Amount (mg) of Vinblastine Sulfate RS, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-length: 262 nm).

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

Column temperature: A constant temperature of about $25^{\circ}C$.

Mobile phase: To 7 mL of diethylamine add water to make 500 mL, and adjust the pH to 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 $20 \,\mu$ L 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 $20 \,\mu\text{L}$ 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, at not exceeding -20° C.

Vinblastine Sulfate for Injection

注射用ビンブラスチン硫酸塩

Vinblastine Sulfate for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of vinblastine sulfate ($C_{46}H_{58}N_4O_9$, H_2SO_4 : 909.05).

Method of preparation Prepare as directed under Injections, with Vinblastine Sulfate.

Description Vinblastine Sulfate for Injection occurs as white to pale yellow, light masses or powder.

It is freely soluble in water.

The pH of a solution (1 in 1000) is 3.5 - 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 sample solution. Pipet 1 mL of the sample solution, add water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 200 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than the main peak from the sample solution is not larger than 1/2 times the peak area of vinblastine from the standard solution, and the total area of the peaks other than the main peak 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 Purity (2) under Vinblastine Sulfate.

System suitability—

Perform as directed in the system suitability in Purity (2) under Vinblastine Sulfate.

Bacterial endotoxins <4.01> Less than 10 EU/mg.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Dissolve 1 Vinblastine Sulfate for Injection in water to make exactly $V \,\text{mL}$ so that each mL contains about 0.4 mg of vinblastine sulfate (C₄₆H₅₈N₄O₉.H₂SO₄), and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Vinblastine Sulfate RS (previously determine the loss on drying in the same conditions 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.

- Amount (mg) of vinblastine sulfate ($C_{46}H_{58}N_4O_9.H_2SO_4$) = $M_S \times A_T/A_S \times 25/V$
- $M_{\rm S}$: Amount (mg) of Vinblastine Sulfate RS, calculated on the dried basis

Foreign insoluble matter $\langle 6.06 \rangle$ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Take an amount of Vinblastine Sulfate for Injection, equivalent to 0.10 g of vinblastine sulfate $(C_{46}H_{58}N_4O_9.H_2SO_4)$, 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 sample solution. Separately, weigh accurately about 10 mg of Vinblastine Sulfate RS (previously determine the loss on drying in the same conditions 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.

- Amount (mg) of vinblastine sulfate ($C_{46}H_{58}N_4O_9.H_2SO_4$) = $M_S \times A_T/A_S \times 10$
- $M_{\rm S}$: Amount (mg) of Vinblastine Sulfate RS, calculated on the dried basis

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant, at 2 to 8°C.

Vincristine Sulfate

ビンクリスチン硫酸塩



C46H56N4O10.H2SO4: 923.04

Methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-acetoxy-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-3azacycloundecino[5,4-*b*]indol-9-yl]-6-formyl-5-hydroxy-8methoxy-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 $C_{46}H_{56}N_4O_{10}$. H_2SO_4 , calculated on the dried basis.

Description Vincristine Sulfate occurs as a white to light yellowish white powder.

It is very soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

Optical rotation $[\alpha]_D^{20}$: +28.5 - +35.5° (0.2 g, calculated on the dried basis, water, 10 mL, 100 mm).

Identification (1) Determine the absorption spectrum of a solution of Vincristine Sulfate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Vincristine Sulfate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Vincristine Sulfate as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Vincristine Sulfate RS: 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 $\langle 1.09 \rangle$ for sulfate.

pH $\langle 2.54 \rangle$ 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 sample solution. Pipet 2 mL of the sample solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly $200 \,\mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area 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 sample 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 sample solution is not larger than 1/4 times the peak area of vincristine from standard solution. Furthermore, the total area of the peaks other than vincristine from the sample 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 4.6 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μ m 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 the pH to 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 after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 12 12 – 27	$\begin{array}{c} 62\\ 62 \rightarrow 92 \end{array}$	$38 \\ 38 \rightarrow 8$

Flow rate: Adjust the flow rate so that the retention time of vincristine is about 15 minutes.

Time span of measurement: About 1.7 times as long as the retention time of vincristine, beginning after the solvent peak.

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 μ L of this solution is equivalent to 1.75 to 3.25% of that from 200 μ L 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 $200 \,\mu$ L 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 $200 \,\mu\text{L}$ 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%.

Loss on drying Perform the test with about 10 mg of Vincristine Sulfate as directed in Method 2 under Thermal Analysis $\langle 2.52 \rangle$ according to the following conditions: not

1566 Vitamin A Oil / Official Monographs

more than 12.0%. *Operating conditions—* Heating rate: 5°C per minute. Temperature range: room temperature to 200°C. Atmospheric gas: dried nitrogen. Flow rate of atmospheric gas: 40 mL per 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 sample solution and the standard solution, respectively. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the vincristine peak areas, $A_{\rm T}$ and $A_{\rm S}$, of both solutions.

Amount (mg) of $C_{46}H_{56}N_4O_{10}$. $H_2SO_4 = M_S \times A_T/A_S$

 $M_{\rm S}$: Amount (mg) of Vincristine Sulfate RS, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 297 nm).

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

Column temperature: A constant temperature of about $25^{\circ}C$.

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 vincristine 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 $10 \,\mu$ L 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 $10 \,\mu\text{L}$ 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 at not exceeding -20° C.

Vitamin A Oil

ビタミン A 油

Vitamin A Oil is synthetic vitamin A esters diluted with fixed oils.

It contains not less than 30,000 vitamin A Units per g.

It may contain suitable antioxidants.

It contains not less than 90.0% and not more than 120.0% of the labeled amount of vitamin A.

Description Vitamin A Oil is a yellow to yellow-brown,

clear or slightly turbid oil. It is odorless or has a faint, characteristic odor.

It is decomposed upon exposure to air or light.

Identification Dissolve Vitamin A Oil, Retinol Acetate RS and Retinol Palmitate RS, equivalent to 15,000 Units, in 5 mL of petroleum ether, and use these solutions as the sample solution, the standard solution (1) and the standard solution (2), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 5 μ L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop with a mixture of cyclohexane and diethyl 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 sample solution has the same color tone and the same *R*f value with the blue spot obtained from the standard solution (2).

Purity (1) Acidity—Dissolve 1.2 g of Vitamin A Oil in 30 mL of a mixture of neutralized ethanol and diethyl 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.

Assay Proceed as directed in Method 1-1 under Vitamin A Assay <2.55>.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and almost well-filled, or under Nitrogen atmosphere.

Vitamin A Oil Capsules

Vitamin A Capsules

ビタミン A 油力プセル

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 requirements 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.

Assay Weigh accurately 20 Vitamin A Oil Capsules, and open the capsules to take out the content. Wash the capsules well with a small amount of diethyl ether, allow the capsules to stand at ordinal temperature to vaporize the diethyl ether, and weigh accurately. Perform the test with the content as directed under Vitamin A Assay <2.55>, and calculate the units of vitamin A per capsule. Before applying Method 1-1, it is necessary to know which the sample is, retinol acetate or retinol palmitate.

Containers and storage Containers-Well-closed contain-

ers. Storage—Light-resistant.

Compound Vitamin B Powder

複方ビタミンB散

Method of preparation

Thiamine Nitrate Riboflavin	10 g 10 g
Pyridoxine Hydrochloride	10 g
Nicotinamide	100 g
Starch, Lactose Hydrate or	
their mixture	a sufficient quantity
	To make 1000 g

Prepare as directed under Powders, with the above ingredients.

Description Compound Vitamin B Powder is orange-yellow in color. It has a slighly bitter taste.

It is slowly affected by light.

Identification (1) Shake 2 g of Compound Vitamin B Powder with 100 mL of water, filter, and to 5 mL of the filtrate add 2.5 mL of sodium hydroxide TS and 0.5 mL of potassium hexacyanoferrate (III) TS. Then add 5 mL of 2methyl-1-propanol, shake the mixture vigorously for 2 minutes, allow to stand, and observe under ultraviolet light: the 2-methyl-1-propanol layer shows a blue-purple fluorescence. This fluorescence disappears when the mixture is acidified, but reappears when it is again made alkaline (thiamine).

(2) Shake 0.1 g of Compound Vitamin B Powder with 100 mL of water, and filter. Perform the following tests with the filtrate (riboflavin).

(i) The filtrate is light yellow-green in color and has an intense yellow-green fluorescence. This color and fluorescence of the solution disappears upon the addition of 0.02 g of sodium hydrosulfite to 5 mL of the filtrate, and again appears by shaking the mixture in air. This fluorescence disappears upon the addition of dilute hydrochloric acid or sodium hydroxide TS.

(ii) To 10 mL of the filtrate placed in a glass-stoppered test tube add 1 mL of sodium hydroxide TS, after illuminating 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 thoroughly with 5 mL of chloroform: the chloroform layer shows yellow-green fluorescence.

(3) Shake 1 g of Compound Vitamin B Powder with 100 mL of diluted ethanol (7 in 10), filter, and to 5 mL of the filtrate add 2 mL of sodium hydroxide TS and 40 mg of manganese dioxide. Heat on a water bath for 30 minutes, cool, and filter. Add 5 mL of 2-propanol to 1 mL of the filtrate, and use the solution as the sample solution. To 3 mL of the sample solution add 2 mL of bartibal buffer solution, 4 mL of 2-propanol and 2 mL of a freshly prepared solution of 2,6-dibromo-N-chloro-1,4-benzoquinone monoimine in ethanol (95) (1 in 4000) prepared when required for use: a blue color develops. To 1 mL of the sample solution add 1 mL of a saturated boric acid solution, and proceed as di-

rected in the same manner as above: no blue color develops (pyridoxine).

(4) Shake 0.5 g of Compound Vitamin B Powder with 10 mL of ethanol (95), filter, and evaporate 1 mL of the filtrate on a water bath to dryness. Add 0.01 g of 2,4-dinitrochlorobenzen to the residue, heat gently for 5-6 seconds to fuse, and after cooling, add 4 mL of potassium hydroxide-ethanol TS: a red color develops (nicotinamide).

(5) Shake 1 g of Compound Vitamin B Powder with 5 mL of diluted ethanol (7 in 10), filter, and use the filtrate as the sample solution. Separately, dissolve 0.01 g each of thiamine mononitrate, riboflavin, pyridoxine hydrochloride and nicotinamide in 1 mL, 50 mL, 1 mL and 1 mL of water, respectively, and use these solutions as standard solutions (1), (2), (3) and (4). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 µL each of the sample 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 chloroform, ethanol (95) and acetic acid (100) (100:50:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (broad spectrum wavelength): four spots from the sample solution show the same color tone and the same Rf value as the corresponding spots from standard solutions (1), (2), (3) and (4).

Containers and storage Containers-Well-closed containers.

Storage-Light-resistant.

Voglibose

ボグリボース

C₁₀H₂₁NO₇: 267.28 3,4-Dideoxy-4-[2-hydroxy-1-(hydroxymethyl)ethylamino]-2-*C*-(hydroxymethyl)-D-*epi*-inositol [*83480-29-9*]

Voglibose contains not less than 99.5% and not more than 101.0% of $C_{10}H_{21}NO_7$, calculated on the anhydrous basis.

Description Voglibose occurs as white crystals or crystalline powder.

It is very slightly soluble in water, freely soluble in acetic acid (100), slightly soluble in methanol, and very slightly soluble in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS.

Identification (1) Determine the infrared absorption spectrum of Voglibose as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

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(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 Spectroscopy $\langle 2.21 \rangle$, using sodium 3-trimethylsilylpropionate-d₄ for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits 2 double signals A at about δ 1.5 ppm, 2 double signals B at about δ 2.1 ppm, 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.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_{D}^{20}$: +45 - +48° (0.2 g calculated on the anhydrous basis, 0.1 mol/L hydrochloric acid TS, 20 mL, 100 mm).

pH $\langle 2.54 \rangle$ Dissolve 1.0 g of Voglibose in 10 mL of water: the pH of the solution is between 9.8 and 10.4.

Melting point <2.60> 163 – 168°C

Purity (1) Heavy metals $\langle 1.07 \rangle$ —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 sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly $50 \,\mu L$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than voglibose obtained from sample solution is not larger than 1/5 times the peak area of voglibose from the standard solution. For the calculate 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. Operating conditions—

Apparatus: Use an apparatus consisting of 2 pumps for the mobile phase and reaction reagent transportation, sample injection port, column, reaction coil, cooling coil, detector and recording device, and the reaction coil and cooling coil maintained at a constant temperature.

Detector: Fluorophotometer (excitation wavelength: 350 nm, fluorescence wavelength: 430 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with pentaethylenehexaaminated polyvinyl alcohol polymer bead for liquid chromatography.

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

Reaction coil: A polytetrafluoroethylene tube 0.5 mm in inside diameter and 20 m in length.

Cooling coil: A polytetrafluoroethylene tube 0.3 mm in inside diameter and 2 m in length.

Mobile phase: To 1.56 g of sodium dihydrogen phosphate dihydrate 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 the mobile phase: Adjust the flow rate so that the retention time of voglibose is about 20 minutes.

Flow rate of the reaction reagent: Same as the flow rate of the mobile phase.

Time span of measurement: About 2.5 times as long as the retention time of voglibose, beginning after the solvent peak. *System suitability*—

Test for required detectability: Pipet 10 mL of the standard solution, and add the mobile phase to make 100 mL. Confirm that the peak area of voglibose obtained from 50 μ L of this solution is equivalent to 7 to 13% of that from 50 μ L of the standard solution.

System performance: When the procedure is run with 50 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the 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 $50 \,\mu\text{L}$ 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%.

Water $\langle 2.48 \rangle$ Not more than 0.2% (0.5 g, coulometric titration).

Residue on ignition $\langle 2.44 \rangle$ 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 $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

> Each mL of 0.1 mol/L perchloric acid VS = $26.73 \text{ mg of } C_{10}H_{21}NO_7$

Containers and storage Containers—Tight containers.

Voglibose Tablets

ボグリボース錠

Voglibose Tablets contain 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 μ m in particle diameter) into a chromatographic column 8 mm in inside diameter and 130 mm in height], 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 diluted ammonia TS (1 in 4) at a rate of about 5 mL per minute. Filter the effluent solution two times through a membrane filter with a pore size not exceeding $0.22 \,\mu\text{m}$. 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 sample solution. Separately, dissolve 20 mg of voglibose 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 $\langle 2.03 \rangle$. Spot 20 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, ammonia water (28) 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 spot from the sample solution and the spot from the standard solution show a yellow-brown color, and the same Rf value.

Uniformity of dosage unit <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Voglibose Tablets add exactly V mL of the mobile phase so that the solution contains about $40 \,\mu\text{g}$ of voglibose (C₁₀H₂₁NO₇) per mL, disintegrate the tablet completely by shaking, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μ m. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Hereinafter, proceed as directed in the Assay.

Amount (g) of voglibose ($C_{10}H_{21}NO_7$) = $M_S \times A_T/A_S \times V/500$

 $M_{\rm S}$: Amount (mg) of voglibose for assay, calculated on the anhydrous basis

Assay To 20 tables of Voglibose Tablets add 80 mL of the mobile phase, and completely disintegrate by shaking. To an exact volume of the solution, equivalent to about 4 mg of voglibose ($C_{10}H_{21}NO_7$), add the mobile phase to make exactly 100 mL, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μ m. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg of voglibose for assay (previously determine the water $\langle 2.48 \rangle$ in the same manner as Voglibose), 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 $50 \,\mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of voglibose.

Amount (mg) of voglibose (
$$C_{10}H_{21}NO_7$$
)
= $M_S \times A_T/A_S \times 1/500$

 $M_{\rm S}$: Amount of voglibose for assay, calculated on the dried basis

Operating conditions—

Apparatus: Use an apparatus consisting of 2 pumps for the mobile phase and reaction reagent transportation, sample injection port, column, reaction coil, cooling coil, detector and recording device, and the reaction coil and cooling coil maintained at a constant temperature.

Detector: Fluorophotometer (excitation wavelength: 350 nm, fluorescence wavelength: 430 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with aminopropylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

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

Reaction coil: A polytetrafluoroethylene tube 0.5 mm in inside diameter and 20 m in length.

Cooling coil: A polytetrafluoroethylene tube 0.3 mm in inside diameter and 2 m in length.

Mobile phase: To 1.56 g of sodium dihydrogen phosphate dihydrate 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 for assay and 0.2 g of lactose monohydrate in 5 mL of water, and add the mobile phase to make 50 mL. When the procedure is run with 50 μ L 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 $50 \,\mu\text{L}$ 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.

Warfarin Potassium

ワルファリンカリウム



 $C_{19}H_{15}KO_4$: 346.42 Monopotassium (1*RS*)-2-oxo-3-(3-oxo-1-phenylbutyl)chromen-4-olate [2610-86-8]

Warfarin Potassium, when dried, contains not less than 98.0% and not more than 102.0% of $C_{19}H_{15}KO_4$.

Description Warfarin Potassium occurs as a white, crystalline powder. It is very soluble in water, and freely soluble in ethanol (95).

It dissolves in sodium hydroxide TS.

The pH of a solution prepared by dissolving 1.0 g of Warfain Potassium in 100 mL of water is 7.2 - 8.3.

It is colored to light yellow by light.

A solution of Warfarin Potassium (1 in 10) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Warfarin Potassium in 0.02 mol/L potassium hydroxide TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Warfarin Potassium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Warfarin Potassium, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum or the spectrum of Warfarin Potassium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Walfarin Potassium (1 in 250) responds to the Qualitative Tests <1.09> (1) for potassium salt.

Purity (1) Alkaline colored substances—Dissolve 1.0 g of Warfarin Potassium in a solution of sodium hydroxide (1 in 20) to make exactly 10 mL, and determine the absorbance at 385 nm within 15 minutes as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, using a solution of sodium hydroxide (1 in 20) as a blank: it does not exceed 0.20.

(2) Heavy metals $\langle 1.07 \rangle$ —Dissolve 2.0 g of Warfarin Potassium in 30 mL of ethanol (95), add 2 mL of dilute acetic acid and ethanol (95) to make 50 mL. 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 ethanol (95) to make 50 mL (not more than 10 ppm).

(3) Related substances—Dissolve 0.10 g of Warfarin Potassium in 100 mL of a mixture of water and methanol (3:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of water and methanol (3:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly $20 \,\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: each peak area other than warfarin obtained with the sample solution is not larger than 1/10 times the peak area of warfarin with the standard solution, and the total area of the peaks other than warfarin is not larger than 1/2 times the peak area of warfarin with the standard solution.

Operating conditions—

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

Time span of measurement: About 2 times as long as the retention time of warfarin beginning after the solvent peak. *System suitability*—

Test for required detectability: To exactly 1 mL of the standard solution add the mixture of water and methanol

(3:1) to make exactly 20 mL. Confirm that the peak area of warfarin obtained with $20 \,\mu\text{L}$ of this solution is equivalent to 3.5 to 6.5% of that with $20 \,\mu\text{L}$ of the standard solution.

System performance: Dissolve 20 mg of propyl parahydroxybenzoate in 50 mL of methanol, and add water to make 200 mL. To 5 mL of this solution add 4 mL of a solution of Warfarin Potassium in the mixture of water and methanol (3:1) (1 in 2000), and add the mixture of water and methanol (3:1) to make 100 mL. When the procedure is run with 20 μ L of this solution under the above operating conditions, propyl parahydroxybenzoate and warfarin are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of warfarin is not more than 2.0%.

Loss on drying $\langle 2.41 \rangle$ Not more than 4.5% (1 g, 105°C, 3 hours).

Assay Weigh accurately about 25 mg each of Warfarin Potassium and Warfarin Potassium RS, previously dried, and separately dissolve in the mixture of water and methanol (3:1) to make exactly 50 mL. Pipet 10 mL each of these solutions, add the mixture of water and methanol (3:1) to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of warfarin.

Amount (mg) of $C_{19}H_{15}KO_4 = M_S \times A_T/A_S$

 $M_{\rm S}$: Amount (mg) of Warfarin Potassium RS

Operating conditions-

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with cyanopropylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

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

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (68:32:1).

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

System suitability-

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

System repeatability: When the test is repeated 6 times with $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of warfarin is not more than 1.0%.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Warfarin Potassium Tablets

ワルファリンカリウム錠

Warfarin Potassium Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of warfarin potassium (C₁₉H₁₅KO₄: 346.42).

Method of preparation Prepare as directed under Tablets, with Warfarin Potassium.

Identification (1) Determine the absorption spectrum of the solution T_2 obtained in the Assay, using 0.02 mol/L potassium hydroxide TS as the blank, as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: it exhibits a maximum between 306 nm and 310 nm, and a minimum between 258 nm and 262 nm. Separately, determine the absorption spectrum of the solution T_1 obtained in the Assay, using 0.02 mol/L hydrochloric acid TS as the blank, as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: it exhibits maxima between 281 nm and 285 nm and between 303 nm and 307 nm, and a minimum between 243 nm and 247 nm.

(2) Weigh a quantity of Warfarin Potassium Tablets, equivalent to 0.01 g of Warfarin Potassium according to the labeled amount, add 10 mL of acetone, shake, and filter. Heat the filtrate on a water bath to evaporate the acetone. To the residue add 10 mL of diethyl ether and 2 mL of dilute hydrochloric acid, and shake: the aqueous layer responds to the Qualitative Tests $\langle 1.09 \rangle$ (1) for potassium salt.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Powder 1 tablet of Warfarin Potassium Tablets, add 40 mL of water, and shake vigorouly for 30 minutes. Add water to make exactly VmL of this solution containing about 20 μ g of warfarin potassium (C₁₉H₁₅KO₄) per ml. Filter this solution, discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 40 mg of Warfarin Potassium RS, previously dried at 105°C for 3 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. Pipet 20 mL each of the sample solution and the standard solution, add 0.05 mol/L hydrochloric acid TS to make exactly 25 mL, and use these solutions as the solution T_1 and the solution S_1 , respectively. Separately, pipet 20 mL each of the sample solution and the standard solution, add 0.05 mol/L potassium hydroxide TS to make exactly 25 mL, and use these solutions as the solution T₂ and the solution S₂, respectively. Determine the absorbances, $A_{\rm T}$ and $A_{\rm S}$, of the solution T_1 and the solution S_1 at 272 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using the solution T_2 and the solution S_2 as the blank, respectively.

Amount (mg) of warfarin potassium ($C_{19}H_{15}KO_4$) = $M_S \times A_T/A_S \times V/2000$

M_S: Amount (mg) of Warfarin Potassium RS

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rates in 15 minutes of 0.5-mg, 1-mg and 2-mg tablet and in 30

minutes of 5-mg tablet of Warfarin Potassium Tablets are not less than 80%.

Start the test with 1 tablet of Warfarin Potassium Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding $0.45 \,\mu\text{m}$. 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 $0.56 \mu g$ of warfarin potassium (C₁₉H₁₅KO₄) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Warfarin Potassium RS, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this 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 exactly 100 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of warfarin from each solution.

Dissolution rate (%) with respect to the labeled amount of warfarin potassium ($C_{19}H_{15}KO_4$)

$$= M_{\rm S} \times A_{\rm T}/A_{\rm S} \times V'/V \times 1/C \times 9/4$$

M_S: Amount (mg) of Warfarin Potassium RS

C: Labeled amount (mg) of warfarin potassium $(C_{19}H_{15}KO_4)$ in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-length: 283 nm).

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

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

Mobile phase: A mixture of methanol, water and phosphoric acid (700:300:1).

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

System suitability-

System performance: When the procedure is run with 100 μ L of the standard solution under the above conditions, the number of theoretical plates and the symmetry factor of the peak of warfarin are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with $100 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of warfarin is not more than 2.0%.

Assay Weigh accurately and powder not less than 20 Warfarin Potassium Tablets. Weigh accurately a portion of the powder, equivalent to about 4 mg of warfarin potassium $(C_{19}H_{15}KO_4)$, add 80 mL of water, shake vigorously for 15 minutes, and add water to make exactly 100 mL. Filter this solution, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 80 mg of Warfarin Potassium RS, previously dried at 105°C for 3 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. Pipet 10 mL each of the sample solution and standard solution, add 0.02 mol/L hydrochloric acid TS to

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make exactly 20 mL, and use these solutions as the solution T_1 and the solution S_1 , respectively. Separately, pipet 10 mL each of the sample solution and standard solution, add 0.02 mol/L potassium hydroxide TS to make exactly 20 mL, and use these solutions as the solution T_2 and the solution S_2 , respectively. Determine the absorbances, A_T and A_S , of the solution T_1 and the solution S_1 at 272 nm as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, using the solution T_2 and the solution S_2 as the blank, respectively.

Amount (mg) of warfarin potassium (C₁₉H₁₅KO₄) = $M_{\rm S} \times A_{\rm T}/A_{\rm S} \times 1/20$

M_S: Amount (mg) of Warfarin Potassium RS

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Water

常水

H₂O: 18.02

Water must meet the Quality Standards of Drinking water provided under the Article 4 of the Water Supply Law (the Ministry of Health, Labour and Welfare Ministerial Ordinance No.101, May 30, 2003). In the case that Water is prepared at individual facilities using well water or industrial water as source water, it must meet the following additional requirement as well as the Quality Standards of Drinking water:

Purity Ammonium <1.02>—Perform the test with 30 mL of Water as directed under Ammonium Limit Test. Prepare the control solution as follows: to 0.15 mL of Standard Ammonium Solution add water for ammonium limit test to make 30 mL (not more than 0.05 mg/L).

Water for Injection

注射用水

Water for Injection is prepared by distillation or by reverse osmosis and/or ultrafiltration, either from Water after applying appropriate pretreatments such as ion-exchange or reverse osmosis, or from Purified Water.

When Water for Injection is prepared by the reverse osmosis and/or ultrafiltration (methods for refining water by using a reverse osmosis membrane module, an ultrafiltration membrane module capable of removing substances having molecular masses of 6,000 and above, or a module using both types of membranes), care must be taken to avoid microbial contamination of the water processing system, and to provide water with equivalent quality to that prepared by distillation consistently.

Water for Injection must be used immediately after preparation. However, it may be stored temporarily, if adequate countermeasures able to prevent microbial proliferation stringently, such as circulating it in a loop at a high temperature, are established.

Description Water for Injection is a clear and colorless liquid, having no odor.

Purity Total organic carbon <2.59>—Not more than 0.50 mg/L.

Conductivity $\langle 2.51 \rangle$ When the test is performed according to the following method, the conductivity (25°C) is not more than 2.1 μ S·cm⁻¹.

Transfer a suitable amount of Water for Injection to a beaker, and stir the water specimen. Adjust the temperature to $25 \pm 1^{\circ}$ C, and begin agitating the water specimen vigorously, while observing its conductivity periodically. When the change in conductivity becomes not greater than $0.1 \,\mu$ S·cm⁻¹ per 5 minutes, adopt the observed value as the conductivity of the water specimen.

Bacterial endotoxins <4.01> Less than 0.25 EU/mL.

Purified Water

精製水

Purified Water is prepared from Water by ionexchange, distillation, reverse osmosis or ultrafiltration, or by a combination of these processes.

It must be used immediately after preparation. However, it may be stored temporarily, if adequate countermeasures for preventing microbial proliferation are taken.

Description Purified Water is a clear and colorless liquid, having no odor.

Purity Total organic carbon <2.59>—Not more than 0.50 mg/L.

Conductivity $\langle 2.51 \rangle$ When the test is performed according to the following method, the conductivity (25°C) is not more than 2.1 μ S·cm⁻¹.

Transfer a suitable amount of Purified Water to a beaker, and stir the water specimen. Adjust the temperature to $25 \pm 1^{\circ}$ C, and begin agitating the water specimen vigorously, while observing its conductivity periodically. When the change in conductivity becomes not greater than $0.1 \,\mu$ S· cm⁻¹ per 5 minutes, adopt the observed value as the conductivity of the water specimen.

Purified Water in Containers

精製水(容器入り)

Purified Water in Containers is prepared from Purified Water by introducing it in a tight container.

It is allowable to describe it as "Purified Water" on the label.

Description Purified Water in Containers is a clear and colorless liquid, having no odor.

Purity Potassium permanganate-reducing substances—To 100 mL of Purified Water in Containers add 10 mL of dilute

sulfuric acid, boil, then add 0.10 mL of 0.02 mol/L potassium permanganate VS, and boil again for 10 minutes: the red color of the solution does not disappear.

Conductivity $\langle 2.51 \rangle$ When the test is performed according to the following method, the conductivity (25°C) is not more than 25 μ S·cm⁻¹ for containers with a nominal volume of 10 mL or less, and not more than 5 μ S·cm⁻¹ for containers with a nominal volume greater than 10 mL.

Transfer a suitable amount of Purified Water in Containers to a beaker, and stir the water specimen. Adjust the temperature to $25 \pm 1^{\circ}$ C, and begin agitating the water specimen vigorously, while observing its conductivity periodically. When the change in conductivity becomes not greater than $0.1 \,\mu\text{S}\cdot\text{cm}^{-1}$ per 5 minutes, adopt the observed value as the conductivity of the water specimen.

Microbial limit $\langle 4.05 \rangle$ The acceptance criteria of TAMC is 10² CFU/mL. Perform the test using soybean-casein digest agar medium.

Containers and storage Containers—Tight containers.

Sterile Purified Water in Containers

滅菌精製水(容器入り)

Sterile Purified Water in Containers is prepared from Purified Water by introducing it into a hermetic container, sealing up the container, then sterilizing the product, or by making it sterile using a suitable method, introducing the sterilized water into a sterile hermetic container by applying aseptic manipulation, then sealing up the container.

Description Sterile Purified Water in Containers is a clear and colorless liquid, having no odor.

Purity Potassium permanganate-reducing substances—To 100 mL of Sterile Purified Water in Containers add 10 mL of dilute sulfuric acid, boil, then add 0.10 mL of 0.02 mol/L potassium permanganate VS, and boil again for 10 minutes: the red color of the solution does not disappear.

Conductivity $\langle 2.51 \rangle$ When the test is performed according to the following method, the conductivity (25°C) is not more than 25 μ S·cm⁻¹ for containers with a nominal volume of 10 mL or less, and not more than 5 μ S·cm⁻¹ for containers with a nominal volume greater than 10 mL.

Transfer a suitable amount of Sterile Purified Water in Containers to a beaker, and stir the water specimen. Adjust the temperature to $25 \pm 1^{\circ}$ C, and begin agitating the water specimen vigorously, while observing its conductivity periodically. When the change in conductivity becomes not greater than 0.1 μ S·cm⁻¹ per 5 minutes, adopt the observed value as the conductivity of the water specimen.

Sterility <4.06> It meets the requirements.

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections can be used in place of hermetic containers.

Sterile Water for Injection in Containers

注射用水(容器入り)

Sterile Water for Injection in Containers is prepared from Water for Injection by introducing it into a hermetic container, sealing up the container, then sterilizing the product, or by making it sterile using a suitable method, introducing the sterilized water into a sterile hermetic container by applying aseptic manipulation, then sealing up the container.

It is allowable to describe it as "Water for Injection" on the label.

For Sterile Water for Injection in Containers prepared from Water for Injection obtained by distillation, an alternative name of "Distilled Water for Injection" may be used.

Description Sterile Water for Injection in Containers is a clear and colorless liquid, having no odor.

Purity Potassium permanganate-reducing substances—To 100 mL of Sterile Water for Injection in Containers add 10 mL of dilute sulfuric acid, boil, then add 0.10 mL of 0.02 mol/L potassium permanganate VS, and boil again for 10 minutes: the red color of the solution does not disappear.

Conductivity $\langle 2.51 \rangle$ When the test is performed according to the following method, the conductivity (25°C) is not more than 25 μ S·cm⁻¹ for containers with a nominal volume of 10 mL or less, and not more than 5 μ S·cm⁻¹ for containers with a nominal volume greater than 10 mL.

Transfer a suitable amount of Sterile Water for Injection in Containers to a beaker, and stir the water specimen. Adjust the temperature to 25 ± 1 °C, and begin agitating the water specimen vigorously, while observing its conductivity periodically. When the change in conductivity becomes not greater than $0.1 \,\mu\text{S}\cdot\text{cm}^{-1}$ per 5 minutes, adopt the observed value as the conductivity of the water specimen.

Bacterial endotoxins <4.01> Less than 0.25 EU/mL.

Extractable volume <6.05> It meets the requirements.

Foreign insoluble matter $\langle 6.06 \rangle$ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> It meets the requirement.

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections can be used in place of hermetic containers.

Weil's Disease and Akiyami Combined Vaccine

ワイル病秋やみ混合ワクチン

Weil's Disease and Akiyami Combined Vaccine is a liquid for injection containing inactivated Weil's disease leptospira, Akiyami A leptospira, Akiyami B leptospira and Akiyami C leptospira.

The product lacking more than a kind of Akiyami leptospira may be prepared, if necessary.

It conforms to the requirements of Weil's Disease and Akiyami Combined Vaccine in the Minimum Requirements for Biological Products.

Description Weil's Disease and Akiyami Combined Vaccine is a white-turbid liquid.

Wheat Starch

Amylum Tritici

コムギデンプン

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (\bullet).

Wheat Starch consists of the starch granules obtained from caryopsis of wheat, *Triticum aestivum* Linné (*Gramineae*).

***Description** Wheat Starch occurs as white masses or powder.

It is practically insoluble in water and in ethanol (99.5). ◆

Identification (1) Examine under a microscope $\langle 5.01 \rangle$ using a mixture of water and glycerinol (1:1), Wheat Starch presents large and small granules, and, very rarely, intermediate sizes. The large granules, usually $10 - 60 \,\mu$ m in diameter, are discoid or, more rarely, reniform when seen face-on. The central hilum and striations are invisible or barely visible and the granules sometimes show cracks on the edges. Seen in profile, the granules are elliptical and fusiform and the hilum appears as a slit along the main axis. The small granules, rounded or polyhedral, are $2 - 10 \,\mu$ m in diameter. Between orthogonally oriented polarizing plates or prisms, the granules show a distinct black cross intersecting at the hilum.

(2) To 1 g of Wheat Starch add 50 mL of water, boil for 1 minute, and allow to cool: a subtle white-turbid, pasty liquid is formed.

(3) To 1 mL of the pasty liquid obtained in (2) add 0.05 mL of diluted iodine TS (1 in 10): a deep blue color is formed, and the color disappears by heating.

pH $\langle 2.54 \rangle$ Put 5.0 g of Wheat Starch in a non-metal vessel, add 25.0 mL of freshly boiled and cooled water, mix gently for 1 minute, and allow to stand for 15 minutes: the pH of the solution is between 4.5 and 7.0.

Purity (1) Iron—To 1.5 g of Wheat Starch add 15 mL of

2 mol/L hydrochloric acid TS, mix, filter, and use the filtrate as the test solution. To 2.0 mL of Standard Iron Solution add water to make 20 mL, and use as the control solution. Put 10 mL each of the test solution and the control solution in test tubes, add 2 mL of a solution of citric acid (2 in 10) and 0.1 mL of mercapto acetic acid, and mix. Alkalize with ammonia solution (28) to litmus paper, add water to make 20 mL, and mix. Transfer 10 mL each of these solutions into test tubes, allow to stand for 5 minutes, and compare the color of these solutions against a white background: the color of the test solution is not more intense than that of the control solution (not more than 10 ppm).

(2) Oxidizing substances—To 4.0 g of Wheat Starch add 50.0 mL of water, shake for 5 minutes, and centrifuge. To 30.0 mL of the supernatant liquid add 1 mL of acetic acid (100) and 0.5 to 1.0 g of potassium iodide, shake, and allow to stand for 25 to 30 minutes at a dark place. Add 1 mL of starch TS, and titrate $\langle 2.50 \rangle$ with 0.002 mol/L sodium thiosulfate VS until the color of the solution disappears. Perform a blank determination and make any necessary correction: the volume of 0.002 mol/L sodium thiosulfate VS consumed is not more than 1.4 mL (not more than 20 ppm, calculated as hydrogen peroxide).

(3) Sulfur dioxide—

(i) Apparatus Use as shown in the figure.



- A: Boiling flask (500 mL)
- B: Funnel (100 mL)
- C: Condenser
- D: Test-tube
- E: Tap

(ii) Procedure Introduce 150 mL of water into the boiling flask, close the tap of the funnel, and pass carbon dioxide through the whole system at a rate of 100 ± 5 mL per minute. Pass cooling water through the condenser, and place 10 mL of hydrogen peroxide-sodium hydroxide TS in the test-tube. After 15 minutes, remove the funnel without interrupting the stream of carbon dioxide, and introduce through the opening into the flask about 25 g of Wheat Starch, accurately weighed, with the aid of 100 mL of water. Apply tap grease to the outside of the connection part of the funnel, and load the funnel. Close the tap of the funnel, pour 80 mL of 2 mol/L hydrochloric acid TS into the funnel, open the tap to introduce the hydrochloric acid into the flask, and close the tap while several mL of the hydrochloric acid remains, in order to avoid losing sulfur dioxide. Place the flask in a water bath, and heat the mixture for 1 hour. Transfer the contents of the test-tube with the aid of a little water to a wide-necked conical flask. Heat in a water bath for 15 minutes, and cool. Add 0.1 mL of bromophenol blue TS, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L sodium hydroxide VS until the color changes from yellow to violet-blue lasting for at least 20 seconds. Perform a blank determination and make any necessary correction. Calculate the amount of sulfur dioxide by applying the following formula: it is not more than 50 ppm.

Amount (ppm) of sulfur dioxide = $V/M \times 1000 \times 3.203$

M: Amount (g) of the sample

V: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed

•(4) Foreign matter. Under a microscope $\langle 5.01 \rangle$, Wheat Starch does not contain starch granules of any other origin. It may contain a minute quantity, if any of fragments of the tissue of the original plant.

Loss on drying $\langle 2.41 \rangle$ Not more than 15.0% (1 g, 130°C, 90 minutes).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.6% (1 g).

◆Containers and storage Containers—Well-closed containers.

White Ointment

白色軟膏

Method of preparation

White Beeswax	50 g
Sorbitan Sesquioleate	20 g
White Petrolatum	a sufficient quantity
	To make 1000 g

Prepare as directed under Ointments, with the above materials.

Description White Ointment is white in color. It has a slight, characteristic odor.

Containers and storage Containers—Tight containers.

Whole Human Blood

人全血液

Whole Human Blood is a liquid for injection which is prepared by mixing human blood cells and an anticoagulant solution for storage.

It conforms to the requirements of Whole Human Blood in the Minimum Requirements for Biological Products.

Description Whole Human Blood is a deep red liquid from which the erythrocytes settle upon standing, leaving a yellow

supernatant layer. A gray layer which mainly consists of leucocytes may appear on the surface of the settled erythrocyte layer. The supernatant layer may become turbid in the presence of fat, or may show the faint color of hemoglobin.

Wine

ブドウ酒

Wine is an alcoholic liquid obtained by fermenting the juice of the fruits of *Vitis vinifera* Linné (*Vitaceae*) or allied plants.

It contains not less than 11 vol% and not more than 14 vol% of ethanol (C_2H_6O : 46.07) (by specific gravity), and not less than 0.10 w/v% and not more than 0.40 w/v% of L-tartaric acid ($C_4H_6O_6$: 150.09).

It contains no artificial sweetener and no artificial coloring agent.

Description Wine is a light yellow or reddish purple to redpurple liquid. It has a characteristic and aromatic odor. It has a slightly astringent and faintly irritating taste.

Specific gravity $\langle 2.56 \rangle$ d_{20}^{20} : 0.990 – 1.010

Optical rotation $\langle 2.49 \rangle$ Boil 160 mL of Wine, neutralize with potassium hydroxide TS, and concentrate to 80 mL on a water bath. Cool, dilute with water to 160 mL, add 16 mL of lead subacetate TS, shake well, and filter. To 100 mL of the filtrate add 10 mL of a saturated solution of sodium sulfate decahydrate, shake well, filter, and use the filtrate as the sample solution. Allow 20 mL of the sample solution to stand for 24 hours, add 0.5 g of activated charcoal, shake, stopper, and allow to stand for 10 minutes. Filter, and observe the optical rotation of the filtrate in a 200-mm cell. Multiply the optical rotation of Wine: it is between -0.3° and $+0.3^{\circ}$.

Purity (1) Total acid [as L-tartaric acid $(C_4H_6O_6)$]—To exactly 10 mL of Wine add 250 mL of freshly boiled and cooled water, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L sodium hydroxide VS (indicator: 1 mL of phenolphthalein TS).

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Each mL of 0.1 mol/L sodium hydroxide VS
= 7.504 \text{ mg of } C_4H_6O_6
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Total acid is not less than $0.40 \ w/v\%$ and not more than $0.80 \ w/v\%.$

(2) Volatile acid [as acetic acid ($C_2H_4O_2$: 60.05)]—Transfer 100 mL of Wine to a beaker, add 1 mL of 1 mol/L sodium hydroxide VS and the same volume of 1 mol/L sodium hydroxide VS as that of 0.1 mol/L sodium hydroxide VS titrated in (1) to make the solution alkaline, and concentrate to 50 mL on a water bath. Cool, add water to make 100 mL, transfer to a 1000-mL distillation flask, containing previously added 100 g of sodium chloride. Wash the beaker with 100 mL of water, and combine the washings in the distillation flask. Add 5 mL of a solution of L-tartaric acid (3 in 20), and distil with steam cautiously to maintain the volume of the solution in the flask until 450 mL of the distillate is obtained for 45 minutes. Dilute the distillate to exactly 500 mL with water, and use this solution as the sample solution. Titrate $\langle 2.50 \rangle$ a 250-mL portion of the sample solution with

0.1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

> Each mL of 0.1 mol/L sodium hydroxide VS = $6.005 \text{ mg of } C_2H_4O_2$

The volatile acid is not more than 0.15 w/v%.

(3) Sulfur dioxide—Stopper a 750-mL round-bottomed flask with a stopper having two holes. Through one hole, insert a glass tube A extending nearly to the bottom of the flask. Through the other hole, insert a glass tube B ending to the neck of the flask. Connect the tube B to a Liebig's condenser, and the end of the condenser to a joint of which inner diameter is 5 mm at the lower end. Connect the other end of the joint with a holed rubber stopper to a U tube having three bulbs as shown in the Figure. Pass carbon dioxide washed with a solution of potassium permanganate (3 in 100) through the tube A. Displace the air in the apparatus by carbon dioxide, and place 50 mL of a freshly prepared and diluted starch TS (1 in 5) and 1 g of potassium iodide in the U tube. From the other end of the U tube, add 1 to 2 drops of 0.01 mol/L iodine VS from a burette. While passing carbon dioxide, remove the stopper of the flask a little, add exactly 25 mL of Wine, 180 mL of freshly boiled and cooled water, 0.2 g of tannic acid, and 30 mL of phosphoric acid, and stopper again. Pass carbon dioxide for further 15 minutes, heat the distillation flask with caution so that 40 to 50 drops of the distillate may be obtained in 1 minute. When the color of starch TS in the U tube is discharged, add 0.01 mol/L iodine VS dropwise from a burette so that the color of the starch TS remains light blue to blue during the distillation. Read the volume of 0.01 mol/L iodine VS consumed when exactly 60 minutes have passed after the beginning of distillation. In this case, however, the coloration of starch TS produced by 1 drop of 0.01 mol/L iodine VS should persist at least for 1 minute.

Each mL of 0.01 mol/L iodine VS = $0.6406 \text{ mg of SO}_2$

The amount of sulfur dioxide (SO₂: 64.06) does not exceed 7.5 mg.



(4) Total sulfuric acid—Transfer 10 mL of Wine to a beaker, boil, and add 50 mL of a solution prepared by dissolving 5.608 g of barium chloride dihydrate in 50 mL of hydrochloric acid and water to make 1000 mL. Cover the beaker, and heat on a water bath for 2 hours, supplying the water lost by distillation. Cool, centrifuge, and decant the supernatant liquid in another beaker. To this solution add 1 to 2 drops of dilute sulfuric acid, and allow to stand for 1 hour: a white precipitate is formed.

(5) Arsenic <1.11>-Evaporate 10 mL of Wine on a

water bath to dryness. Prepare the test solution with the residue according to Method 3, and perform the test (not more than 0.2 ppm).

(6) Glycerin—Pipet 100 mL of Wine into a 150-mL porcelain dish, and concentrate on a water bath to 10 mL. Add 1 g of sea sand (No. 1), and make the solution strongly alkaline by adding a solution prepared by dissolving 4 g of calcium hydroxide in 6 mL of water. Heat on a water bath with constant stirring and pushing down any material adhering to the wall of the dish until the contents of the dish become soft masses. Cool, add 5 mL of ethanol (99.5), and grind to a grue-like substance. Heat on a water bath, add 10 to 20 mL of ethanol (99.5) while agitating, boil, and transfer to a 100-mL volumetric flask. Wash the dish with seven 10-mL portions of hot ethanol (99.5), combine the washings with the contents of the flask, cool, and add ethanol (99.5) to make exactly 100 mL. Filter through a dry filter paper, evaporate 90 mL of the filtrate on a water bath, taking care not to boil the solution during the evaporation. Dissolve the residue in a small amount of ethanol (99.5), transfer to a 50-mL glass-stoppered volumetric cylinder, wash with several portions of ethanol (99.5), and add the washings to the solution in the cylinder to make 15 mL. Add three 7.5-mL portions of dehydrated diethyl ether, shake vigorously each time, and allow to stand. When the solution becomes quite clear, transfer to a tared, flat weighing bottle. Wash the volumetric cylinder with 5 mL of a mixture of dehydrated diethyl ether and ethanol (99.5) (3:2). Transfer the washings to the weighing bottle, and evaporate carefully on a water bath. When the liquid becomes sticky, dry at 105°C for 1 hour, and cool in a desiccator (silica gel), and weigh: the mass of the residue is not less than 0.45 g and not more than 0.90 g.

(7) Reducing sugars—To a 25-mL portion of the sample solution obtained in the Optical rotation add 50 mL of boiling Fehling's TS, and heat for exactly 2 minutes. Filter the separated precipitates by a tared glass filter by suction, wash successively with hot water, with ethanol (95) and with diethyl ether, and continue to dry the precipitates by suction. Heat the filter gently at first, and then strongly until the precipitates become completely black. Cool the precipitates in a desiccator (silica gel), and weigh as copper (II) oxide: the mass of cupric oxide does not exceed 0.325 g.

(8) Sucrose—Transfer a 50-mL portion of the sample solution obtained in the Optical rotation to a 100-mL flask, neutralize with diluted hydrochloric acid (1 in 30), followed by further addition of 5 mL of diluted hydrochloric acid (1 in 30). Heat in a water bath for 30 minutes, cool, neutralize with a solution of potassium hydroxide (1 in 100), add 4 drops of sodium carbonate TS, filter into a 100-mL volumetric flask, wash with water, combine the washings with the filtrate, and add water to make 100 mL. To 25 mL of this solution add 50 mL of boiling Fehling's TS, and proceed as directed in (7), and weigh as copper (II) oxide. From the number obtained by multiplying the mass (g) of copper (II) oxide by 2, deduct the amount (g) of copper (II) oxide determined in (7), and multiply again the number so obtained by 1.2: the number obtained does not exceed 0.104 (g).

(9) Benzoic acid, cinnamic acid and salicylic acid—Transfer exactly 50 mL of the sample solution obtained in (2) to a separator, add 10 g of sodium chloride and 2 mL of dilute hydrochloric acid, and extract with three 10-mL portions of diethyl ether. Combine the diethyl ether extracts, wash with
two 5-mL portions of water, and extract with three 10-mL portions of 0.1 mol/L sodium hydroxide VS. Combine the alkaline extracts, evaporate the diethyl ether by warming on a water bath, cool, neutralize with 1 mol/L hydrochloric acid VS, and add 5 mL of potassium chloride-hydrochloric acid buffer solution and water to make exactly 50 mL. Perform the test as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$ with this solution, using a solution prepared in the same manner instead of the sample solution as the blank: the absorbance does not exceed 0.15 at a wavelength between 220 nm and 340 nm.

(10) Boric acid—Transfer 50 mL of Wine to a porcelain dish, add 5 mL of sodium carbonate TS, evaporate on a water bath to dryness, and ignite: a half portion of the residue does not respond to Qualitative Tests $\langle 1.09 \rangle$ (1) for borate. Dissolve another half portion of the residue in 5 mL of hydrochloric acid: it does not respond to Qualitative Tests $\langle 1.09 \rangle$ (2) for borate.

(11) Methanol—Wine meets the requirements of the Methanol Test $\langle 1.12 \rangle$, when proceeding with exactly 1 mL of ethanol layer obtained by Method 1 of the Alcohol Number Determination $\langle 1.01 \rangle$ and distilling without adding water after shaking with 0.5 g of calcium carbonate.

(12) Formaldehyde—To 25 mL of Wine add 5 g of sodium chloride and 0.2 g of L-tartaric acid, distil, and obtain 15 mL of the distillate. To 5 mL of the distillate add 5 mL of acetyl acetone TS, mix, and heat on a water bath for 10 minutes: the solution has no more color than that of the following control solution.

Control solution: Using 5 mL of water instead of the distillate, perform the test in the same manner.

Extract content 1.9 - 3.5 w/v% Pipet 25 mL of Wine to a 200-mL tared beaker containing 10 g of sea sand (No. 1), previously dried at 105 °C for 2.5 hours, and evaporate to dryness on a water bath. Dry the residue at 105 °C for 2 hours, cool in a desiccator (silica gel), and weigh.

Total ash 0.13 - 0.40 w/v% Pipet 50 mL of Wine to a tared porcelain dish, and evaporate to dryness on a water bath. Ignite the residue to the constant mass, cool, and weigh.

Assay (1) Ethanol—Pipet Wine into a 100-mL volumetric flask at 15°C, transfer to a 300- to 500-mL flask, and wash this volumetric flask with two 15-mL portions of water. Add the washings to the sample in the flask, connect the flask to a distillation tube having a trap, and distil using the volumetric flask as a receiver. When about 80 mL of the distillate is obtained (it takes about 20 minutes), stop the distillation, allow to stand in water at 15°C for 30 minutes, and add water to make exactly 100 mL. Shake well, and determine the specific gravity at 15°C according to Method 3 under Specific Gravity <2.56>: the specific gravity d_{15}^{15} is between 0.982 and 0.985.

(2) L-Tartaric acid—Pipet 100 mL of Wine, add 2 mL of acetic acid (100), 0.5 mL of a solution of potassium acetate (1 in 5) and 15 g of powdered potassium chloride, and shake vigorously to dissolve as much as possible. Add 10 mL of ethanol (95), rub the inner wall of the beaker strongly for 1 minute to induce the crystallization, and allow to stand between 0°C and 5°C for more than 15 hours. Filter the crystals by suction, wash successively the beaker and the crystals with 3-mL portions of a solution prepared by dissolving 15 g

of powdered potassium chloride in 120 mL of diluted ethanol (1 in 6), and repeat the washings five times. Transfer the crystals together with the filter paper to a beaker, wash the filter with 50 mL of hot water, combine the washings in the beaker, and dissolve the crystals by heating. Titrate $\langle 2.50 \rangle$ the solution with 0.2 mol/L sodium hydroxide VS immediately (indicator: 1 mL of phenolphthalein TS). The number obtained by adding 0.75 to the amount (mL) of 0.2 mol/L sodium hydroxide VS consumed represents the amount (mL) of 0.2 mol/L sodium hydroxide VS consumed.

> Each mL of 0.2 mol/L sodium hydroxide VS = $30.02 \text{ mg of } C_4H_6O_6$

Containers and storage Containers—Tight containers.

Wood Creosote

木クレオソート

Wood Creosote is a mixture of phenols obtained from by using wood tar derived from dry distillation of stems and branches of various plants of genus *Pinus* (*Pinaceae*), genus Cryptomeria (Taxodiaceae), genus Fagus (Fagaceae), genus Afzelia (genus Intsia); (Leguminosae), genus Shorea (Dipterocarpaceae) or genus Tectona (Verbenaceae), followed by distillation and collection at 180 to 230°C, then further purification and then re-distillation.

Wood Creosote contains not less than 23.0% and not more than 35% of guaiacol ($C_7H_8O_2$: 124.14).

Description Wood Creosote is a colorless or pale yellow, clear liquid. It has a characteristic odor.

- It is slightly soluble in water.
- It is miscible with methanol and with ethanol (99.5).
- Its saturated solution is acidic.
- It is highly refractive.
- It gradually changes in color by light or by air.

Identification Use the sample solution obtained in the Assay as the sample solution. Separately, dissolve 0.1 g of phenol, *p*-cresol, guaiacol, and 2-methoxy-4-methylphenol in methanol respectively, to make 100 mL. To 10 mL of each solution add methanol to make 50 mL, and use these solutions as standard solution (1), standard solution (2), standard solution (3) and standard solution (4). Perform the test with 10 μ L each of the sample solution, standard solution (1), (2), (3) and (4) as directed under Liquid Chromatography <2.01> according to the following conditions: the main peaks obtained with the sample solution show the same retention times with those obtained with the standard solutions (1), (2), (3) and (4).

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

Specific gravity $\langle 2.56 \rangle$ d_{20}^{20} : not less than 1.076.

Purity (1) Coal Creosote—Accurately measure 10 mL of Wood Creosote, add methanol to make exactly 20 mL, and use this solution as the sample solution. Separately to 1 mg each of benzo[a]pyrene, benz[a]anthracene and dibenz[a,h]anthracene add a small quantity of ethyl acetate,

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if necessary, and add methanol to make 100 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 exactly 1 μ L each of the sample solution and standard solution as directed under Gas Chromatography $\langle 2.02 \rangle$ according to the following conditions: No peaks are detected with the sample solution for the retention times corresponding to benzo[a]pyrene, benz[a]anthracene and dibenz[a,h]anthracene of the standard solution. Change these conditions if any peak is detected for retention times that correspond to benzo[a]pyrene, benz[a]anthracene or dibenz[a,h]anthracene, to verify that such a peak does not belong to benzo[a]pyrene, benz[a]anthracene or dibenz[a,h]anthracene.

Operating conditions—

Detector: A mass spectrometer (EI). Monitored ions:

Benz[a]anthracene: Molecular ion m/z 228, Fragment ion m/z 114	About 14 to 20 minutes
Benzo[a]pyrene: Molecular ion m/z 252, Fragment ion m/z 125	About 20 to 25 minutes
Dibenz[a,h]anthracene: Molecular ion m/z 278, Fragment ion m/z 139	About 25 to 30 minutes

Column: A quartz tube 0.25 mm in inside diameter and 30 m in length, with internal coating $0.25 - 0.5 \,\mu$ m in thickness made of 5% diphenyl-95% dimethyl polysiloxane for gas chromatography.

Column temperature: Inject sample at a constant temperature in vicinity of 45°C, then raise temperature to 240°C at the rate of 40°C per minute, maintain the temperature at 240°C for 5 minutes, then raise temperature to 300°C at the rate of 4°C per minute, then raise the temperature to 320°C at the rate of 10°C per minute, then maintain temperature at 320°C for 3 minutes.

Injection port temperature: A constant temperature in vicinity of 250°C.

Interface temperature: A constant temperature in vicinity of 300°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of benzo[a]pyrene is about 22 minutes.

Split ratio: Splitless.

System suitability—

Test for required detectability: Accurately measure 1 mL of standard solution and add methanol to make exactly 10 mL, and use this solution as the solution for system suitability test. When the test is performed with conditions described above with $1 \,\mu$ L of the solution for system suitability test, the SN ratio of each substance is not less than 3.

System performance: When the procedure is run with conditions described above with $1 \mu L$ of the solution for system suitability test, the elution takes place in order of benz[*a*]anthracene, benzo[*a*]pyrene and then dibenz[*a*,*h*]anthracene.

System repeatability: When the test is repeated 6 times with $1 \mu L$ of the solution for system suitability test under the above conditions, the relative standard deviation of the peak area of benzo[*a*]pyrene, benz[*a*]anthracene and dibenz[*a*,*h*]anthracene is respectively not more than 10%.

(2) Acenaphthene—To 0.12 g of Wood Creosote add

methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of acenaphthene in methanol to make 50 mL. To 5 mL of this solution add methanol to make 20 mL. To 2 mL of this solution add methanol to make 100 mL, and use this solution as the standard solution. Perform the test with exactly 1 μ L each of the sample solution and standard solution as directed under Gas Chromatography $\langle 2.02 \rangle$ according to the following conditions: No peaks are detected with sample solution for the retention time corresponding to acenaphthene of the standard solution. Change these conditions if any peak is detected for the retention time corresponding to acenaphthene, to verify that such a peak does not belong to athenaphthene. *Operating conditions*—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica tube 0.25 mm inside diameter and 60 m in length, with internal coating 0.25. 0.5 μ m in thickness made of polymethylsiloxane for gas chromatography.

Column temperature: Perform injection at a constant temperature in vicinity of 45° C, then raise the temperature by 11.5°C per minute until reaching 160°C, then raise the temperature by 4°C per minute until reaching 180°C, then raise the temperature by 8°C until reaching 270°C, then maintain temperature at 270°C for 3 minutes.

Injection port temperature: 250°C.

Detector temperature: 250°C.

Carrier gas: Helium.

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

Split ratio: Splitless.

System suitability-

Test for required detectability: Accurately measure 1 mL of the standard solution, add methanol to make exactly 10 mL, and use this solution as the solution for system suitability test. When the procedure is run with conditions described above with 1 μ L of solution for system suitability test, the SN ratio of acenaphthene is not less than 3.

System repeatability: When the test is repeated 6 times with $1 \mu L$ of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of acenaphthene is not more than 6.0%.

(3) Other impurities

Add 2 mL of petroleum benzin to 1.0 mL of Wood Creosote, then add 2 mL of barium hydroxide test solution, agitate to mix and allow to stand. No blue or muddy brown color develops in the upper layer of the mixture. Furthermore, no red color develops in the lower layer.

Distilling range $\langle 2.57 \rangle$ 200 – 220°C, not less than 85 vol%.

Assay To about 0.1 g of Wood Creosote, accurately weighed, add methanol to make exactly 50 mL. Pipet 10 mL of this solution add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, add methanol to about 30 mg of accurately measured guaiacol for assay to make exactly 50 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and determine the peak areas, $A_{\rm T}$ and $A_{\rm S}$, of guaiacol for each solution.

Amount (mg) of guaiacol ($C_7H_8O_2$) = $M_S \times A_T/A_S$

M_S: Amount of guaiacol for assay (mg)

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 275 nm).

Column: Fill a stainless steel tube with internal diameter of 4.6 mm and length of 15 cm with $5 \mu m$ of octadecyl-silanized silica gel for gas chromatography.

Column temperature: A constant temperature in vicinity of 40°C.

Mobile phase: Mixture of water and acetonitrile (4:1).

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

System suitability-

System performance: Dissolve 2 mg each of guaiacol and phenol in methanol to make 10 mL. The procedure is run with conditions described above with $10 \,\mu$ L of this solution, the elution takes place in order of phenol then guaiacol, with the degree in separation of not less than 2.5.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of guaiacol is not more than 1.5%.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Xylitol

キシリトール

C₅H₁₂O₅: 152.15 *meso*-Xylitol [*87-99-0*]

Xylitol, when dried, contains not less than 98.0% of $C_5H_{12}O_5$.

Description Xylitol occurs as white crystals or powder. It is odorless and has a sweet taste.

It is very soluble in water, slightly soluble in ethanol (95). It 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): blue-green color is produced without turbidity.

(2) Determine the infrared absorption spectrum of Xylitol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH $\langle 2.54 \rangle$ 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 <2.60> 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 $\langle 1.03 \rangle$ —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 $\langle 1.14 \rangle$ —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 $\langle 1.07 \rangle$ —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 $\langle 1.11 \rangle$ —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 in 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 copper (I) oxide. Remove the 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 <2.50> with 0.02 mol/L potassium permanganate VS: not more than 1.0 mL of 0.02 mol/L potassium permanganate VS is consumed.

Loss on drying <2.41> Not more than 1.0% (1 g, in vacuum, phosphorus (V) oxide, 24 hours).

Residue on ignition $\langle 2.44 \rangle$ 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 mL of this solution into an iodine flask, add 50 mL of potassium periodate TS exactly, and heat in a water bath for 15 minutes. After cooling, add 2.5 g of potassium iodide, stopper, shake well, allow to stand for 5 minutes in a dark place, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium thiosulfate VS = $1.902 \text{ mg of } C_5 H_{12} O_5$

Containers and storage Containers—Tight containers.

Xylitol Injection

キシリトール注射液

Xylitol Injection is an aqueous solution for injection.

It contains not 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.

Description Xylitol Injection is a clear, colorless liquid. It 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 sample solution. Separately, dissolve 0.1 g of xylitol in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thinlayer Chromatography $\langle 2.03 \rangle$. Spot 2 μ L each of the sample solution and 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 sample solution and standard solution show a blackish brown color and the same *R*f value.

pH <2.54> 4.5 - 7.5

Bacterial endotoxins <4.01> Less than 0.50 EU/mL.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: 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, and add water to make exactly 250 mL. Measure exactly 10 mL of this solution, and add water to make exactly 100 mL. Then, pipet 10 mL of this solution into an iodine flask, and proceed as directed in the Assay under Xylitol.

Each mL of 0.1 mol/L sodium thiosulfate VS = $1.902 \text{ mg of } C_5 H_{12} O_5$

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Dried Yeast

乾燥酵母

Dried Yeast is dried and powdered cells of yeast belonging to *Saccharomyces*.

It contains not less than 400 mg of protein and not less than 100 μ g of thiamine compounds [as thiamine chloride hydrochloride (C₁₂H₁₇ClN₄OS.HCl: 337.27)] in each 1 g.

Description Dried Yeast occurs as a light yellowish white to brown powder. It has a characteristic odor and taste.

Identification Dried Yeast, when examined under a microscope $\langle 5.01 \rangle$, shows isolated cells, spheroidal or oval in shape, and 6 to 12 μ m 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 $\langle 5.01 \rangle$: no or only a few granules are tinted blackish purple.

Loss on drying $\langle 2.41 \rangle$ Not more than 8.0% (1 g, 100°C, 8 hours).

Total ash $\langle 5.01 \rangle$ Not more than 9.0% (1 g).

Assay (1) Protein—Weigh accurately about 50 mg of Dried Yeast and perform the test as directed under Nitrogen Determination $\langle 1.08 \rangle$.

Amount (mg) of protein in 1 g of Dried Yeast = $N \times 6.25 \times 1/M$

N: Amount (mg) of nitrogen (N) M: 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°C to 85°C for 30 minutes with occasional shaking. After cooling, add water to make exactly 100 mL, and centrifuge for 10 minutes. Pipet 4 mL of the 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°C to 50°C 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 to $110 \,\mu\text{m}$ in particle diameter) into a chromatographic tube about 1 cm in inside diameter and about 17 cm in length, and elute at the flow rate 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 per minute, and combine the eluate. To the eluate add exactly 1 mL of the internal standard solution and 0.01 g of sodium 1-octanesulfonate, and after dissolving, use this solution as the sample solution. Separately, weigh accurately about 15 mg of Thiamine Chloride Hydrochloride RS (previously determine the water $\langle 2.48 \rangle$ in the same manner as Thiamine Chloride Hydrochloride), dissolve in 0.001 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 1 mL of this solution, and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add exactly 1 mL of the internal standard solution and 3 mL of the mobile phase, and use this solution as the standard solution. Perform the test with 200 μ L each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of thiamine to that of the internal standard.

Amount (
$$\mu$$
g) of thiamine in 1 g of Dried Yeast
= $M_S/M_T \times Q_T/Q_S \times 12.5$

- $M_{\rm S}$: Amount (mg) of Thiamine Chloride Hydrochloride RS, calculated on the anhydrous basis
- $M_{\rm T}$: Amount (g) of the sample

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 inside diameter and 15 to 30 cm in length, packed with octadecyl-silanized silica gel for liquid chromatography (5 to $10 \,\mu$ m in particle diameter).

Column temperature: A constant temperature of 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 $200 \,\mu\text{L}$ of the standard solution under the above operating conditions, and calculate the resolution. 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.

Containers and storage Containers-Tight containers.

Zaltoprofen

ザルトプロフェン



C₁₇H₁₄O₃S: 298.36 (*2RS*)-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 $C_{17}H_{14}O_3S$.

Description Zaltoprofen occurs as white to light yellow, crystals or crystalline powder.

It is freely soluble in acetone, soluble in methanol and in ethanol (99.5), and practically insoluble in water.

It 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 to melt, and then carbonize. After cooling, add 5 mL of diluted hydrochloric acid (1 in 2): the gas evolved darkens moisten lead (II) acetate paper.

(2) Determine the absorption spectrum of a solution of Zaltoprofen in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Zaltoprofen as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 135 – 139°C

Purity (1) Heavy metals $\langle 1.07 \rangle$ —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 $\langle 1.11 \rangle$ —Prepare the test solution with 1.0 g of Zaltoprofen according to Method 3, using 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (2 in 25), and perform the test (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 sample solution. Pipet 1 mL of the sample 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 exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area 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 sample 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 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m 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.

Time span of measurement: About 15 times as long as the retention time of zaltoprofen beginning after the solvent peak.

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 zaltoprofen obtained with 20 μ L of this solution is equivalent to 8 to 12% of that

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with $20 \,\mu\text{L}$ of the standard solution.

System performance: Dissolve 25 mg of zaltoprofen and 50 mg of isopropyl benzoate in 100 mL of ethanol (99.5). Pipet 1 mL of this solution, and add the mobile phase to make exactly 50 mL. When the procedure is run with $20 \,\mu$ L 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.

System repeatability: When the test is repeated 6 times with $20 \,\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of zaltoprofen is not more than 2.0%.

Loss on drying $\langle 2.41 \rangle$ Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Zaltoprofen, dissolve in 50 mL of methanol, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, 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

ザルトプロフェン錠

Zaltoprofen Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of zaltoprofen (C₁₇H₁₄O₃S: 298.36).

Method of preparation Prepare as directed under Tablets, with Zaltoprofen.

Identification Powder a suitable amount of Zaltoprofen Tablets. To a portion of the powder, equivalent to 80 mg of Zaltoprofen, add 30 mL of ethanol (99.5), shake well, and centrifuge. To 1 mL of the 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 $\langle 2.24 \rangle$: it exhibits maxima between 227 nm and 231 nm and between 329 nm and 333 nm, and a shoulder between 241 nm and 245 nm.

Uniformity of dosage units $\langle 6.02 \rangle$ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Zaltoprofen Tablets add 4 mL of water, and shake to disintegrate. Add a suitable amount of ethanol (95), shake, then add ethanol (95) to make exactly V mL so that each mL contains about 4 mg of zaltoprofen (C₁₇H₁₄O₃S), and centrifuge. Pipet 2 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution and ethanol (95) to make 50 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of zaltoprofen (C₁₇H₁₄O₃S)
=
$$M_{\rm S} \times Q_{\rm T}/Q_{\rm S} \times V/20$$

$M_{\rm S}$: Amount (mg) of zaltoprofen for assay

Internal standard solution—A solution of benzyl benzoate in acetonitrile (1 in 1000).

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Zaltoprofen Tablets is not less than 75%.

Start the test with 1 tablet of Zaltoprofen Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding $0.45 \,\mu\text{m}$. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about $44 \,\mu g$ of zaltoprofen (C17H14O3S) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of zaltoprofen for assay, previously dried at 105°C for 4 hours, dissolve in 20 mL of ethanol (99.5), and add the dissolution medium to make exactly 100 mL. Pipet 4 mL of this solution, add the dissolution medium to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, $A_{\rm T}$ and $A_{\rm S}$, of the sample solution and standard solution at 340 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using the dissolution medium as the control.

Dissolution rate (%) with respect to the labeled amount of zaltoprofen ($C_{17}H_{14}O_3S$)

$$= M_{\rm S} \times A_{\rm T}/A_{\rm S} \times V'/V \times 1/C \times 180$$

 $M_{\rm S}$: Amount (mg) of zaltoprofen for assay

C: Labeled amount (mg) of zaltoprofen for assay in 1 tablet

Assay To 10 tablets of Zaltoprofen Tablets add 40 mL of water, shake to disintegrate, then add a suitable amount of ethanol (95), shake, add ethanol (95) to make exactly 200 mL, and centrifuge. Pipet an amount of the 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 sample solution. Separately, weigh accurately about 80 mg of zaltoprofen for assay, previously dried at 105°C for 4 hours, add 4 mL of water and ethanol (95) to make exactly 20 mL. Pipet 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 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of zaltoprofen to that of the internal standard.

> Amount (mg) of zaltoprofen (C₁₇H₁₄O₃S) = $M_{\rm S} \times Q_{\rm T}/Q_{\rm S} \times 1/10$

 $M_{\rm S}$: Amount (mg) of zaltoprofen for assay

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 4.6 mm in inside diame-

ter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m 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 $5 \,\mu L$ 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 $5 \mu L$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of zaltoprofen to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Zidovudine

ジドブジン



C₁₀H₁₃N₅O₄: 267.24 3'-Azido-3'-deoxythymidine [*30516-87-1*]

Zidovudine contains not less than 97.0% and not more than 102.0% of $C_{10}H_{13}N_5O_4$, calculated on the anhydrous basis.

Description Zidovudine occurs as a white to pale yellowish white powder.

It is freely soluble in methanol, soluble in ethanol (99.5), and sparingly soluble in water.

It gradually turns yellow-brown on exposure to light. Melting point: about 124°C.

Identification Determine the infrared absorption spectrum of Zidovudine as directed in the potassium bromide disc method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Zidovudine RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve the sample and the RS separately in a small amount of water and dry them in a desiccator (in vacuum, phosphorus (V) oxide), and perform the test with the residues.

Optical rotation $\langle 2.49 \rangle$ [α]_D²⁵: +60.5 - +63.0° (0.5 g calculated on the anhydrous basis, ethanol (99.5), 50 mL, 100 mm).

Purity (1) Heavy metals $\langle 1.07 \rangle$ — Proceed with 1.0 g of Zidovudine 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) 1-[(2R,5S)-2,5-Dihydro-5-(hydroxymethyl)-2-furyl]thymine, triphenylmethanol, and other related substances-Dissolve 0.20 g of Zidovudine in methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, add 1 mL of the sample solution to 20 mg each of thymine for liquid chromatography, 1-[(2R,5S)-2,5-dihydro-5-(hydroxymethyl)-2-furyl]thymine for thin-layer chromatography, and triphenylmethanol for thin-layer chromatography, and add methanol to dissolve to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thinlayer Chromatography $\langle 2.03 \rangle$. Spot 10 μ L each of the sample solution and 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 (9:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot obtained from the sample solution that corresponds to the position of the 1-[(2R,5S)-2,5-dihydro-5-(hydroxymethyl)-2-furyl]thymine obtained from the standard solution is not more intense than the spot from the standard solution, and the spot other than the principal spot and spots other than thymine and 1-[(2R,5S)-2,5-dihydro-5-(hydroxymethyl)-2-furyl]thymine from the sample solution is not more intense than zidovudine spot from the standard solution. However, the 3 spots from the standard solution appear in ascending order of Rf value thymine, 1-[(2R,5S)-2,5dihydro-5-(hydroxymethyl)-2-furyl]thymine, and zidovudine. Furthermore, spray evenly on the plate a solution of vanillin in sulfuric acid (1 in 100): the spot from the sample solution corresponding to the spot of triphenylmethanol from the standard solution is not more intense than the spot from the standard solution.

(3) Thymine, 3'-chloro-3'-deoxythymidine, and other related substances-Use the sample solution obtained in the Assay as the sample solution. Separately, weigh accurately about 20 mg of thymine for liquid chromatography, dissolve in 100 mL of methanol, and add the mobile phase to make exactly 250 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 $10 \,\mu L$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the thymine peak areas, $A_{\rm T}$ and $A_{\rm S}$, of the sample and standard solutions, and calculate the amount of thymine using the following formula: the amount is not more than 2.0%. Also, determine the peak area of each peak obtained from the sample solution by the automatic integration method, and calculate the amounts of related substances other than thymine by the area percentage method: the amount of 3'-chloro-3'-deoxythymidine, whose relative retention time to zidovudine is 1.2, is not more than 1.0%, and is not more than 0.5% for all other related substances. Finally, the total amount of thymine, 3'-chloro-3'deoxythymidine, and all related substances obtained above is not more than 3.0%.

Amount (%) of thymine = $M_{\rm S}/M_{\rm T} \times A_{\rm T}/A_{\rm S} \times 10$

 $M_{\rm S}$: Amount (mg) of thymine for liquid chromatography $M_{\rm T}$: Amount (mg) of Zidovudine

Operating conditions—

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

Time span of measurement: About 2 times as long as the retention time of zidovudine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the sample solution, add the mobile phase 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 to make exactly 20 mL. Confirm that the peak area of zidovudine obtained from 10 μ L of this solution is equivalent to 3.5 to 6.5% of that from 10 μ L of the solution for system suitability test.

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

Water $\langle 2.48 \rangle$ Not more than 1.0% (0.25 g, coulometric titration).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.2% (0.5 g).

Assay Weigh accurately about 50 mg of Zidovudine and Zidovudine RS (separately determine the water <2.48> using the same manner as Zidovudine), and dissolve in the mobile phase to make exactly 50 mL. Pipet 10 mL of each solution, add the mobile phase to make them exactly 50 mL, and use these solutions as the sample and the standard solutions, respectively. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas of zidovudine, $A_{\rm T}$ and $A_{\rm S}$ of both solutions.

Amount (mg) of $C_{10}H_{13}N_5O_4 = M_S \times A_T/A_S$

 $M_{\rm S}$: Amount (mg) of Zidovudine RS, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 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 (particle diameter: 5μ m).

Column temperature: A constant temperature of about $25^{\circ}C$.

Mobile phase: A mixture of water and methanol (4:1).

Flow rate: Adjust the flow rate so that the retention time of zidovudine is about 15 minutes.

System suitability-

System performance: Dissolve 50 mg of Zidovudine in 50 mL of the mobile phase. Separately, dissolve 5 mg of 3'-chloro-3'-deoxythymidine for liquid chromatography in 50 mL of the mobile phase. Mix 10 mL and 1 mL of these solutions, respectively, and add the mobile phase to make 50 mL. When the procedure is run with 10 μ L of this solution under the above conditions, zidovudine and 3'-chloro-3'-deoxythymidine are eluted in this order with the resolution between these peaks being not less than 1.4, and the symmetry factor of the peak of zidovudine is not more than 1.5.

System repeatability: When the test is repeated 6 times with $10 \,\mu\text{L}$ of the standard solution under the above conditions, the relative standard deviation of the peak area of zidovudine is not more than 2.0%.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Zinc Chloride

塩化亜鉛

ZnCl₂: 136.29

Zinc Chloride contains not less than 97.0% of $ZnCl_2$.

Description Zinc Chloride occurs as white, crystalline powder, rods, or masses. It is odorless.

It is very soluble in water, and freely soluble in ethanol (95), and its solution may sometimes be slightly turbid. The solution becomes clear on addition of a small amount of hydrochloric acid.

The pH of an aqueous solution of Zinc Chloride (1 in 2) is between 3.3 and 5.3.

It is deliquescent.

Identification An aqueous solution of Zinc Chloride (1 in 30) responds to the Qualitative Tests <1.09> for zinc salt and chloride.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Zinc Chloride in 10 mL of water and 2 drops of hydrochloric acid: the solution has no color, and is clear.

(2) Sulfate $\langle 1.14 \rangle$ —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%).

(3) 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.

(4) 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.

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 (not more than 50 ppm).

(5) Alkali earth metals and alkali metals—Dissolve 2.0 g of Zinc Chloride in 120 mL of water, add ammonium sulfide TS to complete precipitation, 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 mass is not more than 10.0 mg.

(6) Arsenic $\langle 1.11 \rangle$ —Prepare the test solution with 0.40 g of Zinc Chloride according to Method 1, and perform the test (not more than 5 ppm).

(7) Oxychloride—Shake gently 0.25 g of Zinc Chloride

with 5 mL of water and 5 mL of ethanol (95), and add 0.3 mL of 1 mol/L hydrochloric acid VS: the solution is clear.

Assay Weigh accurately about 0.3 g of Zinc Chloride, 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 $\langle 2.50 \rangle$ with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 1.363 mg of ZnCl₂

Containers and storage Containers-Tight containers.

Zinc Oxide

酸化亜鉛

ZnO: 81.38

Zinc Oxide, when ignited, contains not less than 99.0% of ZnO.

Description Zinc Oxide occurs as a white, amorphous powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95), in acetic acid (100) and in diethyl ether.

It dissolves in diute hydrochloric acid and in sodium hydroxide TS.

It gradually absorbs carbon dioxide from air.

Identification (1) Heat Zinc Oxide strongly: a yellow color develops on strong heating, and disappears on cooling.

(2) A solution of Zinc Oxide in dilute hydrochloric acid (1 in 10) responds to the Qualitative Tests <1.09> for zinc salt.

Purity (1) Carbonate, and clarity and color of solution—Mix 2.0 g of Zinc Oxide with 10 mL of water, add 30 mL of dilute sulfuric acid, and heat on a water bath with stirring: no effervescence occurs, and the solution obtained is clear and colorless.

(2) Alkalinity—To 1.0 g of Zinc Oxide add 10 mL of water, and boil for 2 minutes. Cool, 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: no color develops.

(3) Sulfate $\langle 1.14 \rangle$ —Shake 0.5 g of Zinc Oxide with 40 mL of water, and filter. Take 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 diluted hydrochloric acid (1 in 2), dissolve 0.1 g of ammonium peroxodisulfate in this solution, and extract with 20 mL of 4methyl-2-pentanone. Add 30 mL of acetic acid-sodium acetate buffer solution for Iron Limit Test, pH 4.5, to the 4methyl-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 L-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 (95) 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 back: the color of the liquid from the test solution is not stronger than that from the control solution (not more than 10 ppm).

(5) 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 on a water bath until solution is complete. Cool, and add 5 drops of potassium chromate TS: no turbidity is produced.

(6) Arsenic $\langle 1.11 \rangle$ —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 ignitiono20 $\langle 2.43 \rangle$ 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 exactly 100 mL. Pipet 10 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 $\langle 2.50 \rangle$ with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 4.069 mg of ZnO

Containers and storage Containers—Tight containers.

Zinc Oxide Oil

チンク油

Zinc Oxide Oil contains not less than 45.0% and not more than 55.0% of zinc oxide (ZnO: 81.38).

Method of preparation

Zinc Oxide	500 g
Fixed oli	a sufficient quantity
	To make 1000 g

Mix the above ingredients. An appropriate quantity of Castor Oil or polysorbate 20 may be used partially in place of fixed oil.

Description Zinc Oxide Oil is a white to whitish, slimy substance, separating a part of its ingredients when stored for a prolonged period.

Identification Mix thoroughly, and place 0.5 g of Zinc Oxide Oil in a crucible, heat gradually raising the temperature until the mass is thoroughly charred, and then ignite it strongly: a yellow color is produced, and disappears on cooling. To the residue add 10 mL of water and 5 mL of dilute hydrochloric acid, shake well, and filter. To the filtrate add

1586 Zinc Oxide Ointment / Official Monographs

2 to 3 drops of potassium hexacyanoferrate (II) TS: a white precipitate is formed (zinc oxide).

Assay Weigh accurately about 0.8 g of Zinc Oxide Oil, mixed well, place in a crucible, heat gradually raising the temperature until the mass is thoroughly charred, and then ignite until the residue becomes yellow, and cool. Dissolve the residue in 1 mL of water and 1.5 mL of hydrochloric acid, and add water to make exactly 100 mL. Pipet 20 mL of this solution, add 80 mL of water, and add a solution of so-dium hydroxide (1 in 50) until a small amount of precipitates begins to form in the solution. Add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate $\langle 2.50 \rangle$ with 0.05 mol/L disodium dihydrogen ethylene-diamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 4.069 mg of ZnO

Containers and storage Containers—Tight containers.

Zinc Oxide Ointment

亜鉛華軟膏

Zinc Oxide Ointment contains not less than 18.5% and not more than 21.5% of zinc oxide (ZnO: 81.38).

Method of preparation

Zinc Oxide Liquid Paraffin	200 g 30 g
White Ointment	a sufficient quantity
	To make 1000 g

Prepare as directed under Ointments, with the above ingredients. White Beeswax, Sorbitan Sesquioleate or White Petrolatum may be used instead of White Ointment.

Description Zinc Oxide Ointment is white in color.

Identification Place 1 g of Zinc Oxide Ointment in a crucible, melt by warming, heat gradually raising the temperature until the mass is thoroughly charred, and then ignite it strongly: a yellow color is produced, and disappears on cooling. To the residue add 10 mL of water and 5 mL of dilute hydrochloric acid, shake well, and filter. To the filtrate add 2 to 3 drops of potassium hexacyanoferrate (II) TS: a white precipitate is formed (zinc oxide).

Purity Calcium, magnesium and other foreign inorganic matters—Place 2.0 g of Zinc Oxide Ointment in a crucible, melt by warming, and heat gradually raising the temperature, until the mass is thoroughly charred. Ignite the mass strongly until the residue becomes uniformly yellow, and cool. Add 6 mL of dilute hydrochloric acid, and heat on a water bath for 5 to 10 minutes: the solution is colorless and clear. Filter the solution, add 10 mL of water to the filtrate, and add ammonia TS until the precipitate first formed redissolves. Add 2 mL each of ammonium oxalate TS and disodium hydrogenphosphate TS to this solution: the solution remains unchanged or becomes very slightly turbid within 5 minutes.

Assay Weigh accurately about 2 g of Zinc Oxide Ointment, place in a crucible, melt by warming, heat gradually raising the temperature until the mass is thoroughly charred, and then ignite until the residue becomes uniformly yellow, and cool. Dissolve the residue in 1 mL of water and 1.5 mL of hydrochloric acid, and add water to make exactly 100 mL. Add 80 mL of water to exactly 20 mL of this solution, and add a solution of sodium hydroxide (1 in 50) until a small amount of precipitates begins to form in the solution. Add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate $\langle 2.50 \rangle$ with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 4.069 mg of ZnO

Containers and storage Containers—Tight containers.

Zinc Oxide Starch Powder

亜鉛華デンプン

Method of preparation

Zinc Oxide	500 g
Starch	a sufficient quantity

To make 1000 g

Prepare as directed under Powders, with the above ingredients.

Description Zinc Oxide Starch Powder occurs as a white powder.

Identification (1) Place 1 g of Zinc Oxide Starch Powder in a crucible, heat gradually, raising the temperature until it is charred, and then ignite strongly: a yellow color develops, and disappears on cooling. To the residue add 10 mL of water and 5 mL of dilute hydrochloric acid, shake well, and filter. To the filtrate add 2 to 3 drops of potassium hexacyanoferrate (II) TS: a white precipitate is formed (zinc oxide).

(2) Shake well 1 g of Oxide Starch Powder with 10 mL of water and 5 mL of dilute hydrochloric acid, and filter. Boil the residue on a filter paper with 10 mL of water, cool, and add 1 drop of iodine TS: a dark blue-purple color is produced (starch).

Containers and storage Containers—Tight containers.

Zinc Sulfate Hydrate

硫酸亜鉛水和物

ZnSO₄.7H₂O: 287.55

Zinc Sulfate Hydrate contains not less than 99.0% and not more than 102.0% of $ZnSO_4.7H_2O$.

Description Zinc Sulfate Hydrate occurs as colorless crystals or white crystalline powder.

It is very soluble in water, and very slightly soluble in

JP XVI

ethanol (99.5).

It effloresces in dry air.

Identification (1) A solution of Zinc Sulfate Hydrate (1 in 20) responds to the Qualitative Tests <1.09> for zinc salt.

(2) A solution of Zinc Sulfate Hydrate (1 in 20) responds to the Qualitative Tests <1.09> for sulfate.

pH <2.54> Dissolve 1.0 g of Zinc Sulfate Hydrate in 20 mL of water: the pH of the solution is between 4.4 and 6.0.

Purity (1) Clarity and color of solution—Dissolve 0.25 g of Zinc Sulfate Hydrate in 5 mL of water: the solution is clear and colorless.

(2) Heavy metals $\langle 1.07 \rangle$ —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.

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 (not more than 10 ppm).

(3) Alkali earth metals and alkali metals—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 Residue on Ignition $\langle 2.44 \rangle$: the mass of the residue is not more than 5.0 mg.

(4) Arsenic $\langle 1.11 \rangle$ —Prepare the test solution with 1.0 g of Zinc Sulfate Hydrate according to Method 1, and perform the test (not more than 2 ppm).

Loss on drying $\langle 2.41 \rangle$ Not less than 35.5% and not more than 38.5% (1 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 <2.50> with 0.01 mol/L disodium dihydorogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 2.876 mg of ZnSO₄.7H₂O

Containers and storage Containers—Tight containers.

Zinc Sulfate Ophthalmic Solution

硫酸亜鉛点眼液

Zinc Sulfate Ophthalmic Solution contains not less than 0.27 w/v% and not more than 0.33 w/v% of zinc sulfate hydrate (ZnSO₄.7H₂O: 287.55).

Method of preparation

Zinc Sulfate Hydrate	3 g
Boric Acid	20 g
Sodium Chloride	5 g
Fennel Oil	2 mL
Purified Water or Purified	
Water in Containers	a sufficient quantity
	To make 1000 mL

Prepare as directed under Ophthalmic Solution, with the above ingredients.

Description Zinc Sulfate Ophthalmic Solution is a clear, colorless liquid.

Identification (1) Zinc Sulfate Ophthalmic Solution responds to the Qualitative Tests $\langle 1.09 \rangle$ for zinc salt.

(2) Zinc Sulfate Ophthalmic Solution responds to the Qualitative Tests $\langle 1.09 \rangle$ for borate.

(3) Zinc Sulfate Ophthalmic Solution responds to the Qualitative Tests $\langle 1.09 \rangle$ for chloride.

Assay Pipet accurately 25 mL of Zinc Sulfate Ophthalmic Solution, add 100 mL of water and 2 mL of ammoniaammonium chloride buffer solution, pH 10.7, and titrate $\langle 2.50 \rangle$ with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

> Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 2.876 mg of ZnSO₄.7H₂O

Containers and storage Containers—Tight containers.

Zinostatin Stimalamer

ジノスタチン スチマラマー

Chromophore moiety



(4S,6R,11R,12R)-11- $[\alpha$ -D-2,6-Dideoxy-2-(methylamino)galactopyranosyloxy]-4-[(4R)-2-oxo-1,3-dioxolan-4-yl]-5oxatricyclo[8.3.0.0^{4,6}]trideca-1(13),9-diene-2,7-diyn-12-yl 2-hydroxy-7-methoxy-5-methylnaphthalene-1-carboxylate

Apoprotein molety bonded to styrene-maleic acid alternate copolymer

R-AAPTATVTPS SGLSDGTVVK VAGAGLQAGT AYDVGQCAWV DTGVLACNPA DFSSVTADAD GSASTSLTVR RSFEGFLFDG TRWGTVDCTT AACQVGLSDA AGNGPEGVAI SFN



R¹ and R², and R¹¹ and R² are different each other as follows, respectively.



A¹=H or NH₄ A², A³=H, NH₄ or C₄H₉ (no C₄H₉ appears at the same time at A² and A³) Average m+n=about 5.5

[123760-07-6]

Zinostatin Stimalamer consists 1 molecule of zinostatin, consisting of chromophore and apoprotein (polypeptide consisting of 113 amino acid residues) and 2 molecules of partially butyl-esterified styrene-maleic acid alternate copolymer, and has average molecular mass of about 15,000. The alternate copolymer is bound an amido bond to α -amino group of alanine of N-terminal and to ε -amino group of lysine 20 of the apoprotein.

It contains not less than $900 \mu g$ (potency) and not more than $1080 \mu g$ (potency) per mg, calculated on the anhydrous basis. The potency of Zinostatin Stimalamer is expressed as mass (potency) of zinostatin stimalamer.

Description Zinostatin Stimalamer occurs as a pale yellow powder.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

Identification (1) Dissolve 10 mg of Zinostatin Stimalamer in 1 mL of sodium hydroxide TS, and add a drop of copper (II) sulfate TS: a purple color develops.

(2) Dissolve 1 mg of Zinostatin Stimalamer in 1 mL of 0.05 mol/L phosphate buffer solution, pH 7.0, add 0.5 mL of a solution of trichloroacetic acid (1 in 5), and shake: a white precipitate is formed.

(3) Determine the absorption spectra of solutions of Zinostatin Stimalamer and Zinostatin Stimalamer RS in 0.05 mol/L phosphate buffer solution, pH 7.0 (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectra of Zinostatin Stimalamer and Zinostatin Stimalamer RS as directed in the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance $\langle 2.24 \rangle = E_{1 \text{ cm}}^{1\%}$ (268 nm): 15.5 – 18.5 (4 mg calculated on the anhydrous basis, 0.05 mol/L phosphate buffer solution, pH 7.0, 10 mL).

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_D^{20}$: $-30.0 - -38.0^\circ$ (20 mg calculated on the anhydrous basis, 0.05 mol/L phosphate buffer solution, pH 7.0, 5 mL, 100 mm).

pH <2.54> Dissolve 10 mg of Zinostatin Stimalamer in 1 mL of water: the pH of the solution is between 4.5 and 5.5.

Purity (1) Clarity and color of solution—Dissolve 20 mg of Zinostatin Stimalamer in 2 mL of 0.05 mol/L phosphate buffer solution, pH 5.0: the solution is clear, and the absorbance at 400 nm of this solution after addition of 3 mL of 0.05 mol/L phosphate buffer solution, determined as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, is not more than 0.25.

(2) Heavy metals $\langle 1.07 \rangle$ —Weigh accurately 40 mg of Zinostatin Stimalamer, place in a crucible, carbonize and incinerate according to Method 2, add 2 mL of hydrochloric acid, and evaporate on a water bath to dryness. After cooling, weigh the residue $M_{\rm T}$ g. Then, moisten the residue with 0.1 mL of diluted hydrochloric acid (1 in 5), add 1 mL of water, 85 μ L of diluted ammonia TS (1 in 2) and 0.1 mL of dilute acetic acid, and add water so that the mass is $M_{\rm T}$ + 2.0 g. Adjust the pH of this solution to 3.2 to 3.4 with diluted ammonia TS (1 in 20) or diluted hydrochloric acid (1 in 50), add water so that the mass is $M_{\rm T}$ + 2.5 g, and use this solution as the test solution. Separately, prepare the blank solution in the same manner without the sample. Separately, take 2 mL of nitric acid, 5 drops of sulfuric acid and 2 mL of hydrochloric acid, and evaporate to dryness according to Method 2. After cooling, weigh the residue $M_{\rm S}$ g. Then, moisten the residue with 0.1 mL of diluted hydrochloric acid (1 in 5), and proceed in the same manner as directed in the preparation of the test solution. After adjusting the pH of the solution so obtained to 3.2 to 3.4, add 80 μ L of Standard Lead Solution, and add water so that the mass is $M_{\rm S}$ + 2.5 g, and use this solution as the control solution. Add $10 \,\mu\text{L}$ each of diluted sodium sulfide TS (1 in 6) to the test solution, the blank solution and the control solution, mix, and allow to stand for 5 minutes. Determine the absorbances, $A_{\rm T}$, $A_{\rm O}$ and $A_{\rm S}$ of the test solution, the blank solution and the control solution at 400 nm as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: $A_{\rm T} - A_{\rm O}$ is not larger than $A_{\rm S} - A_{\rm O}$ (not more than 20 ppm).

(3) Styrene-maleic acid alternating copolymer partial butyl ester and neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3)—

(i) Test solutions

Solution A: Dissolve 36.6 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 48 mL of 1 mol/L hydrochloric acid TS, 0.23 mL of N,N,N',N'-tetramethylethylenediamine and water to make 100 mL.

Solution B: Dissolve 33.3 g of acrylamide and 0.89 g of N,N'-methylenebisacrylamide in water to make 100 mL. Preserve in a cold place, avoiding exposure to light.

Solution C: Dissolve 5.98 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 48 mL of 1 mol/L hydrochloric acid TS, 0.46 mL of N,N,N',N'-tetramethylethylenediamine and water to make 100 mL.

Solution D: Dissolve 10.0 g of acrylamide and 2.5 g of N,N'-methylenebisacrylamide in water to make 100 mL. Preserve in a cold place, avoiding exposure to light.

Solution E: Dissolve 4 mg of riboflavin in water to make 100 mL. Preserve in a cold place, avoiding exposure to light.

Solution F: Dissolve 3.0 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 14.4 g of glycine in water to make 500 mL.

Buffer solution for sample: To 50 mL of Solution C add 20 mL of water and 10 mL of glycerin solution (3 in 5).

(ii) Gels

Resolving gel: Mix 2.5 mL of Solution A and 7.5 mL of Solution B. Mix the mixture with 10 mL of freshly prepared ammonium peroxodisulfate solution (7 in 5000) after degassing under reduced pressure. Pour this mixture into a glass tube, 5 mm in inside diameter and 10 cm in length, to make 7 cm height, put water gently on the upper surface of the mixture, and allow to polymerize for 60 minutes. After polymerization, remove the water from the upper surface of the gel.

Stacking gel: Mix 1 mL of Solution C, 2 mL of Solution D, 1 mL of Solution E and 4 mL of water, pour 0.2 mL of the mixture on the resolving gel, put water gently on the upper surface of the mixture, and allow to polymerize under a fluorescent light for 60 minutes. After polymerization, remove the water from the upper surface of the gel.

(iii) Standard solution Weigh accurately about 6 mg of styrene-maleic acid alternating copolymer partial butyl ester, calculated on the anhydrous basis, and dissolve in the buffer solution for sample to make exactly 20 mL. Separately, weigh accurately about 6 mg of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3), and dissolve in the buffer solution for sample to make exactly 20 mL. Pipet 1 mL each of these solutions, add the buffer solution for sample to make exactly 20 mL, and use this solution as the standard solution.

(iv) Sample solution Weigh accurately about 5 mg of Zinostatin Stimalamer, calculated on the anhydrous basis, dissolve in the buffer solution for sample to make exactly 10 mL.

(v) Procedure Mount the gel in the electrophoresis apparatus. Add a mixture of 200 mL of Solution F and 2 mL of bromophenol blue solution (1 in 100,000) to the top reser-

voir (cathode) and 300 mL of Solution F to the lower reservoir (anode). Introduce carefully exactly 100 μ L each of the sample solution and standard solution onto the surface of separate gels, and allow electrophoresis at room temperature to take place with a current of 2 mA per tube as a bromophenol blue band is passing in the stacking gel and then increase the current to 4 mA per tube as the bromophenol blue band is passing in the resolving gel, and stop the current when the band reached at 5 cm from the upper end of the gel.

(vi) Staining and decolorization Dissolve 0.1 g of Coomassie brilliant blue G-250 in 100 mL of trichloroacetic acid solution (1 in 2), and mix before using 1 volume of this solution and 2 volumes of water. Immerse the gels for 15 hours in this mixture, and transfer into about 20 mL of acetic acid (100) solution (7 in 100) to remove the excess of dye. Replace the acetic acid (100) solution until the back ground of the gel becomes colorless.

(vii) Determination Determine the peak areas, A_{T1} , A_{T2} , A_{S1} and A_{S2} , of styrene-maleic acid alternating copolymer partial butyl ester and neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3) obtained from the sample solution and the standard solution, based on the absorbance at 600 nm of the gels determined by using a densitometer. Calculate the amounts of styrene-maleic acid alternating copolymer partial butyl ester and neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester mulae: their amounts are not more than 3.0%, respectively.

Amount (%) of styrene-maleic acid alternating copolymer partial butyl ester

 $= M_{\rm S1}/M_{\rm T} \times A_{\rm T1}/A_{\rm S1} \times 5/2$

Amount (%) of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3) = $M_{S2} \times (P_S/100)/M_T \times A_{T2}/A_{S2} \times 5/2$

- M_{S1} : Amount (mg) of styrene-maleic acid alternating copolymer partial butyl ester, calculated on the anhydrous basis
- M_{S2} : Amount (mg) of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3), calculated on the anhydrous basis
- $M_{\rm T}$: Amount (mg) of sample, calculated on the anhydrous basis
- $P_{\rm S}$: Purity (%) of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3)

(4) Neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (1:1)-Weigh accurately about 10 mg of Zinostatin Stimalamer, calculated on the anhydrous basis, dissolve in the mobile phase to make exactly 1 mL, and use this solution as the sample stock solution. Separately, weigh accurately about 10 mg of neocarzinostatin (separately determine the water $\langle 2.48 \rangle$ in the same manner as Zinostatin Stimalamer), dissolve in the mobile phase to make exactly 50 mL, and use this solution as the standard stock solution. Pipet 0.2 mL each of the sample stock solution and the standard stock solution, add to them exactly 1.5 mL each of a solution, prepared by dissolving 38.1 g of sodium tetraborate decahydrate in dilute sodium hydroxide TS to make 1000 mL, add exactly 1.2 mL of a solution of sodium 2,4,6-trinitrobenzenesulfonate (1 in 20), allow to stand for 10 minutes at room temperature, then add

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exactly 6 mL of sodium sulfite-sodium dihydrogen phosphate TS, shake thoroughly, and use these solutions as the sample solution and the standard solution, respectively. Separately, pipet 0.2 mL of the sample stock solution, add 1.5 mL of a solution, prepared by dissolving 38.1 g of sodium tetraborate decahydrate in dilute sodium hydroxide TS to make 1000 mL, add exactly 1.2 mL of water, allow to stand for 10 minutes at room temperature, then add exactly 6 mL of sodium sulfite-sodium dihydrogen phosphate TS, shake thoroughly, and use this solution as the blank solution. Perform the test with exactly 0.25 mL each of the sample solution, the standard solution and the blank solution as directed under Liquid Chromatography <2.01> under the following conditions, and determine the peak area, $A_{\rm T}$, of trinitrobenzenesulfonic acid derivative of neocarzinostatinstyrene-maleic acid alternating copolymer partial butyl ester condensate (1:1) obtained from the sample solution, the peak area, $A_{\rm S}$, of trinitrobenzenesulfonic acid derivative of neocarzinostatin obtained from the standard solution, which retention time is almost the same as that of trinitrobenzenesulfonic acid derivative of neocarzinostatin-styrenemaleic acid alternating copolymer partial butyl ester condensate (1:1) obtained from the sample solution, and the peak area, A_0 , obtained from the blank solution. Calculate the amount of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (1:1) by the following formula: not more than 5.0%.

Amount (%) of neocarzinostatin-styrene-maleic acid

alternating copolymer partial butyl ester condensate (1:1) = $M_{\rm S}/M_{\rm T} \times (A_{\rm T} - A_0)/A_{\rm S} \times 2 \times 2.280$

- $M_{\rm S}$: Amount (mg) of neocarzinostatin calculated on the anhydrous basis
- $M_{\rm T}$: Amount (mg) of sample, calculated on the anhydrous basis

Operating conditions—

Detector: A visible absorption photometer (wavelength: 436 nm).

Column: Pre-column is a stainless steel column 7.5 mm in inside diameter and 7.5 cm in length, packed with silica gel for liquid chromatography (10 μ m in particle size). Separation column is a stainless steel column 7.5 mm in inside diameter and 60 cm in length, packed with silica gel for liquid chromatography (10 μ m in particle size), which is coupled to the pre-column.

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

Mobile phase: Dissolve 3.78 g of potassium dihydrogen phosphate and 5.52 g of anhydrous disodium hydrogen phosphate in water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of trinitrobenzenesulfonic acid derivative of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (1:1) is about 21 minutes.

System suitability—

System performance: When the procedure is run with 0.25 mL of the standard stock solution under the above operating conditions excepting at 254 mm, the number of theoretical plates and the symmetry factor of the peak of neocarzinostatin are not less than 2000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 3 times

with 0.25 mL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trinitrobenzenesulfonic acid derivative of neocarzinostatin is not more than 10%.

(5) Manufacturing process origin inorganic salts—Being specified separately.

Water <2.48> Not more than 12.0% (10 mg, coulometric titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics $\langle 4.02 \rangle$ according to the following conditions. Perform the procedures of (iii), (iv) and (v) without exposure to direct or indirect sunlight.

(i) Test organism-Micrococcus luteus ATCC 9341

(ii) Culture medium—Use the medium i in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.9 to 8.1 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Zinostatin Stimalamer RS equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 50 mL, and use this solution as the high concentration standard solution. Pipet 5 mL of the high concentration standard solution, add 0.1 mol/L phosphate buffer solution, pH 8.0, to make exactly 20 mL, and use this solution as the low concentration standard solution.

(iv) Sample solutions—Weigh accurately an amount of Zinostatin Stimalamer equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 50 mL, and use this solution as the high concentration sample solution. Pipet 5 mL of the high concentration sample solution, add 0.1 mol/L phosphate buffer solution, pH 8.0, to make exactly 20 mL, and use this solution as the low concentration sample solution.

(v) Procedure—Allow to stand at 3 to 5° C for 2 hours before incubation.

Containers and storage Containers—Tight containers. Storage—Light-resistant, and not exceeding -20° C.

Zolpidem Tartrate

ゾルピデム酒石酸塩



 $(C_{19}H_{21}N_{3}O)_{2}$. $C_{4}H_{6}O_{6}$: 764.87 N,N,6-Trimethyl-2-(4methylphenyl)imidazo[1,2-*a*]pyridine-3-acetamide hemi-(2*R*,3*R*)-tartrate [99294-93-6]

Zolpidem Tartrate contains not less than 98.5% and not more than 101.0% of $[(C_{19}H_{21}N_3O)_2.C_4H_6O_6]$, calculated on the anhydrous basis.

Description Zolpidem Tartrate occurs as a white, crystal-

line powder.

It is freely soluble in acetic acid (100), soluble in N,Ndimethylformamide and in methanol, sparingly soluble in water, and slightly soluble in ethanol (99.5) and in acetic anhydride.

It dissolves in 0.1 mol/L hydrochloric acid TS.

It gradually changes to yellow in color on exposure to light.

Optical rotation $[\alpha]_D^{20}$: about +1.8° (1 g, *N*,*N*-dimethyl-formamide, 20 mL, 100 mm).

Identification (1) Dissolve 50 mg of Zolpidem Tartrate in 5 mL of acetic acid (100) and add 3 drops of Dragendorff's TS: an orange precipitate is formed.

(2) Determine the absorption spectrum of a solution of Zolpidem Tartrate in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Zolpidem Tartrate as directed in the potassium bromide disk method under the Infrared Spectrophotometry $\langle 2.25 \rangle$, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Zolpidem Tartrate in methanol (1 in 10) responds to the Qualitative Tests <1.09> (3) for tartrate.

Purity (1) Heavy metals $\langle 1.07 \rangle$ —Proceed with 2.0 g of Zolpidem Tartrate 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 10 mg of Zolpidem Tartrate in 20 mL of methanol and use this solution as the sample solution. Pipet 1 mL of the sample 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 exactly 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: each area of the peak other than the peak of zolpidem from the sample solution is not larger than the peak area of zolpidem from the standard solution.

Operating conditions—

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

Column: A stainless steel tube 4.6 mm in inside diameter and 7.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

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

Mobile phase: To 4.9 g of phosphoric acid add 1000 mL of water, and adjust the pH to 5.5 with triethylamine. To 11 volumes of this solution add 5 volumes of methanol and 4 volumes of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of zolpidem is about 5 minutes.

Time span of measurement: About 5 times as long as the retention time of zolpidem.

System suitability—

System performance: Dissolve 10 mg each of Zolpidem

Tartrate and benzyl parahydroxybenzoate in 100 mL of methanol. When the procedure is run with 5 μ L of this solution under the above operating conditions, zolpidem and benzyl parahydroxybenzoate 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 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of zolpidem is not more than 5.0%.

(3) Residual solvent Being specified separately.

Water $\langle 2.48 \rangle$ Not more than 3.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition $\langle 2.44 \rangle$ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Zolpidem Tartrate, dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3) and titrate $\langle 2.50 \rangle$ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = $38.24 \text{ mg of } (C_{19}H_{21}N_3O)_2.C_4H_6O_6$

Containers and storage Containers—Tight containers. Storage conditions—Light-resistant.

Zolpidem Tartrate Tablets

ゾルピデム酒石酸塩錠

Zolpidem Tartrate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of zolpidem tartrate $[(C_{19}H_{21}N_3O)_2.C_4H_6O_6:$ 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, shake for 30 minutes, and filter. Discard the first 20 mL of the filtrate, 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 TS to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 235 nm and 239 nm and between 292 nm and 296 nm.

Uniformity of dosage units $\langle 6.02 \rangle$ Perform the test according to the following method: it meets the requirements 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 disintegrate the tablet by shaking for 15 minutes. Add 2V/5 mL of methanol, then add exactly V/10 mL of the internal standard solution, shake for 15 minutes, and add methanol to make V mL so that each mL contains about 0.1 mg of zolpidem tartrate $[(C_{19}H_{21}N_3O)_2.C_4H_6O_6]$. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of zolpidem tartrate for assay (separately determine the water $\langle 2.48 \rangle$ in the same manner as

Zolpidem Tartrate), and dissolve in 25 mL of 0.1 mol/L hydrochloric acid TS, add exactly 25 mL of the internal standard solution, then add methanol to make 250 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay.

Amount (mg) of zolpidem tartrate [($C_{19}H_{21}N_3O$)₂. $C_4H_6O_6$] = $M_S \times Q_T/Q_S \times V/250$

 $M_{\rm S}$: Amount (mg) of zolpidem tartrate for assay, calculated on the anhydrous basis

Internal standard solution—A solution of benzyl parahydroxybenzoate in methanol (1 in 1000).

Dissolution $\langle 6.10 \rangle$ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Zolpidem Tartrate Tablets is not less than 80%.

Start the test with 1 tablet of Zolpidem Tartrate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding $0.45 \,\mu\text{m}$. 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 μ g of zolpidem tartrate [(C19H21N3O)2.C4H6O6] according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of zolpidem tartrate for assay (separately determine the water <2.48> in the same manner as Zolpidem Tartrate), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, and add water to make exactly 200 mL. Pipet 25 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, $A_{\rm T}$ and $A_{\rm S}$, of the sample solution and standard solution at 242 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using diluted the 2nd fluid for dissolution test (1 in 2) as the blank.

Dissolution rate (%) with respect to the labeled amount

of zolpidem tartrate [(C₁₉H₂₁N₃O)₂.C₄H₆O₆]

 $= M_{\rm S} \times A_{\rm T}/A_{\rm S} \times V'/V \times 1/C \times 45/4$

- $M_{\rm S}$: Amount (mg) of zolpidem tartrate for assay, calculated on the anhydrous basis
- C: Labeled amount (mg) of zolpidem tartrate $[(C_{19}H_{21}N_3O)_2.C_4H_6O_6]$ in 1 tablet

Assay To 20 Zolpidem Tartrate Tablets add V/10 mL of 0.1 mol/L hydrochloric acid TS, and disintegrate the tablet by shaking for 15 minutes. Add 2V/5 mL of methanol, then add exactly V/10 mL of the internal standard solution, shake for 15 minutes, and add methanol to make VmL so that each mL contains about 1 mg of zolpidem tartrate $[(C_{19}H_{21}N_{3}O)_{2}.C_{4}H_{6}O_{6}]$. Centrifuge this solution, add to 1 mL of the 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 sample solution. Separately, weigh accurately about 25 mg of zolpidem tartrate for assay (separately determine the water $\langle 2.48 \rangle$ in the same manner as Zolpidem Tartrate), and dissolve in 25 mL of 0.1 mol/L hydrochloric acid TS, add exactly 2.5 mL of the internal standard solution, then add methanol to make 250 mL, and use this solution as the standard solution. Perform the test with

 $5 \,\mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.0I \rangle$, and calculate the ratios, $Q_{\rm T}$ and $Q_{\rm S}$, of the peak area of zolpidem to that of the internal standard.

Amount (mg) of zolpidem tartrate $[(C_{19}H_{21}N_{3}O)_{2}.C_{4}H_{6}O_{6}]$ in 1 tablet of Zolpidem Tartrate Tablets $= M_{\rm S} \times Q_{\rm T}/Q_{\rm S} \times V/500$

 $M_{\rm S}$: Amount (mg) of zolpidem tartrate for assay, calculated on the anhydrous basis

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 4.6 mm in inside diameter and 7.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

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

Mobile phase: To 4.9 g of phosphoric acid add 1000 mL of water, and adjust to pH 5.5 with triethylamine. To 550 mL of this solution add 250 mL of methanol and 200 mL of acetonitrile.

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 $5 \mu L$ 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 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio 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.