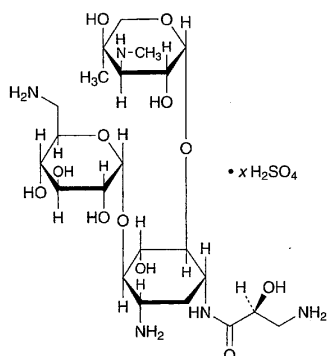


## Isepamicin Sulfate

硫酸イセパマイシン



$C_{22}H_{43}N_5O_{12} \cdot xH_2SO_4$

*O*-6-Amino-6-deoxy- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-*O*-[3-deoxy-4-*C*-methyl-3-methylamino- $\beta$ -L-arabinopyranosyl-(1 $\rightarrow$ 6)]-2-deoxy-1-*N*-[(2*S*)-3-amino-2-hydroxypropanoyl]-D-streptamine sulfate [67814-76-0]

Isepamicin Sulfate contains not less than 670  $\mu$ g (potency) per mg, calculated on the anhydrous basis. The potency of Isepamicin Sulfate is expressed as mass (potency) of isepamicin ( $C_{22}H_{43}N_5O_{12}$ ; 569.60).

**Description** Isepamicin Sulfate occurs as a white to pale yellowish white powder.

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

It is hygroscopic.

**Identification** (1) Dissolve 0.02 g of Isepamicin Sulfate in 1 mL of water, add 3 mL of anthrone TS, shake, and allow to stand: a blue-purple color develops.

(2) Dissolve 0.01 g each of Isepamicin Sulfate and Isepamicin Sulfate Reference Standard in 5 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under the Thin-layer chromatography. Spot 5  $\mu$ L each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ammonia water (28), ethanol (99.5), 1-butanol and chloroform (5:5:4:2) to a distance of about 15 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at about 100°C for about 10 minutes: the principal spots from the sample solution and the standard solution exhibit a red-brown color and show the same *R<sub>f</sub>* value.

(3) Dissolve 0.01 g of Isepamicin Sulfate in 1 mL of water, and add 1 drop of barium chloride TS: a white precipitate is produced.

**Optical rotation**  $[\alpha]_D^{20}$ : +100 – +120° (0.25 g calculated on the anhydrous bases, water, 25 mL, 100 mm).

**pH** Dissolve 0.5 g of Isepamicin Sulfate in 5 mL of water: the pH of the solution is between 5.5 and 7.5.

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Isepamicin Sulfate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals—Proceed with 1.0 g of Isepamicin Sulfate according to Method 4, and perform the test. Prepare the control solution with 1.0 mL Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Perform the test with 5  $\mu$ L of the sample solution obtained in the Assay as directed under the Assay. Determine each peak area of the sample solution by the automatic integration method, and calculate the amounts of their peaks by the area percentage method: the amount of HAPA-gentamine-B equivalent to about 0.4 of the relative retention time to isepamicin is not more than 5%, and gentamicin B equivalent to about 1.3 of that is not more than 3%. Correct the peak area of gentamicin B by multiplying the sensitivity coefficient, 1.11.

**Operating conditions**—

Apparatus, detector, column, column temperature, reaction coil, mobile phase, reagent, reaction temperature, flow rate of the mobile phase, and flow rate of the reagent: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of isepamicin.

**System suitability**—

Test for required detection: Pipet 1 mL of the standard solution, add water to make exactly 10 mL, and use this solution as the solution for the test for required detection. Pipet 1 mL of the solution, and add water to make exactly 10 mL. Confirm that the peak area of isepamicin obtained from 5  $\mu$ L of this solution is equivalent to 7 to 13% of that obtained from 5  $\mu$ L of the solution for the test for required detection.

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

**Water** Not more than 12.0% (0.2 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination).

**Residue on ignition** Not more than 1.0% (1 g).

**Assay** Weigh accurately an amount of Isepamicin Sulfate and Isepamicin Sulfate Reference Standard, equivalent to about 0.02 g (potency), dissolve each in water to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5  $\mu$ L each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the peak areas,  $A_T$  and  $A_S$ , of isepamicin of the solutions.

Amount [ $\mu$ g (potency)] of isepamicin ( $C_{22}H_{43}N_5O_{12}$ )  
= amount [mg (potency)] of Isepamicin Sulfate

$$\text{Reference Standard} \times \frac{A_T}{A_S} \times 1000$$

**Operating conditions**—

Apparatus: Consist of two pumps for the mobile phase and the reagent transport, inject port, column, reaction coil, detector and recorder. Use a reaction coil with thermostat.

Detector: Fluorometry (excitation wavelength: 360 nm, detection wavelength: 440 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  $\mu$ m in particle diameter).

Column temperature: A constant temperature of about

25°C.

Reaction coil: A column 0.25  $\mu\text{m}$  in inside diameter and 5 m in length.

Mobile phase: Dissolve 28.41 g of anhydrous sodium sulfate and 5.23 g of sodium 1-pentane sulfonate in 900 mL of water, add 1 mL of acetic acid (100), and add water to make exactly 1000 mL.

Reagent: To 500 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 10.0, add 5 mL of a solution of *o*-phthalaldehyde in ethanol (95) (2 in 25), 1 mL of 2-mercaptoethanol and 2 mL of a solution of lauromacrogol (1 in 4).

Reaction temperature: A constant temperature of about 45°C.

Flow rate of the mobile phase: About 0.6 mL per minute.

Flow rate of the reagent: About 0.5 mL per minute.

System suitability—

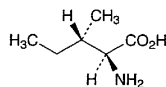
System performance: Dissolve 2 mg of Gentamicin B in 10 mL of the standard solution. When the procedure is run with 5  $\mu\text{L}$  of this solution under the above operating conditions, isepamicin and gentamicin B are eluted in this order with the resolution between these peaks being not less than 1.0.

System repeatability: When the test is repeated 5 times with 5  $\mu\text{L}$  of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of isepamicin is not more than 3%.

**Containers and storage** Containers—Tight containers.

## L-Isoleucine

L-イソロイシン



$\text{C}_6\text{H}_{13}\text{NO}_2$ : 131.17

(2*S*,3*S*)-2-Amino-3-methylpentanoic acid [73-32-5]

L-Isoleucine, when dried, contains not less than 98.5% of  $\text{C}_6\text{H}_{13}\text{NO}_2$ .

**Description** L-Isoleucine occurs as white crystals or crystalline powder. It is odorless or has a faint characteristic odor, and has a slightly bitter taste.

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

It dissolves in dilute hydrochloric acid.

**Identification** Determine the infrared absorption spectrum of L-Isoleucine, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation**  $[\alpha]_{\text{D}}^{20}$ : +39.5 – +41.5° (after drying, 1 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

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

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

(2) Chloride—Perform the test with 0.5 g of L-Isoleucine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate—Perform the test with 0.6 g of L-Isoleucine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium—Perform the test with 0.25 g of L-Isoleucine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals—Dissolve 1.0 g of L-Isoleucine in 40 mL of water and 2 mL of dilute acetic acid by warming, cool, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(6) Arsenic—Prepare the test solution with 1.0 g of L-Isoleucine according to Method 2, and perform the test using Apparatus B (not more than 2 ppm).

(7) Other amino acids—Dissolve 0.10 g of L-Isoleucine 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 the Thin-layer Chromatography. Spot 5  $\mu\text{L}$  each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and 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 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** Not more than 0.30% (1 g, 105°C, 3 hours).

**Residue on ignition** Not more than 0.10% (1 g).

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

Each mL of 0.1 mol/L perchloric acid VS  
= 13.117 mg of  $\text{C}_6\text{H}_{13}\text{NO}_2$

**Containers and storage** Containers—Tight containers.

## Isoniazid

イソニアジド

