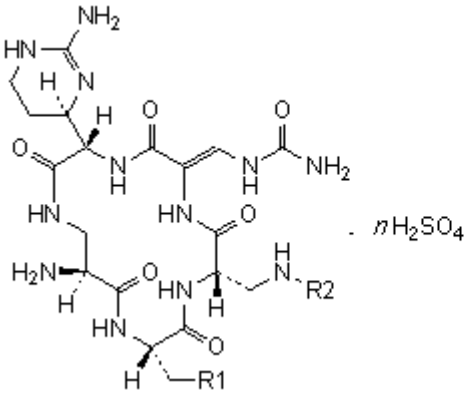
Capreomycin sulfate (Capreomycini sulfas)

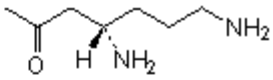
2020-01

*[**Note from the Secretariat.** The user should note that the monograph describes a chromatographic assay to determine the mass concentrations of capreomycin IA, IB, IIA and IIB in a sample under investigation. These concentrations should comply with the requirements set out under the heading "Definition". The pharmaceutical substance also has to be tested with a suitably validated microbiological assay method and the obtained result has to comply with appropriate limits for the activity of capreomycin. A correlation between the mass concentrations of IA, IB, IIA and IIB and the activity of the substance, determined with microbiological methods, has not been established during the elaboration of the monograph.]*



. $n H_2SO_4$

β-Lysyl =



Component	R1	R2
Capreomycin IA	OH	β-Lysyl
Capreomycin IB	H	β-Lysyl
Capreomycin IIA	OH	H
Capreomycin IIB	H	H

Capreomycin (base)	IA	IB	IIA	IIB
Molecular formula	$C_{25}H_{44}N_{14}O_8$	$C_{25}H_{44}N_{14}O_7$	$C_{19}H_{32}N_{12}O_7$	$C_{19}H_{32}N_{12}O_6$
Relative molecular mass	668.7	652.7	540.5	524.5
CAS Reg. no.	37280-35-6	33490-33-4	62639-89-8	62639-90-1
Theoretical value of *n* in neutral sulfate salt	2	2	1.5	1.5

Chemical names

Capreomycin IA: sulfate salt of ([(*Z*){(3*S*,9S,12*S*,15*S*)-15-amino-3-[(4*R*)-2-amino-1,4,5,6-tetrahydropyrimidin-4-yl]-9-({[(3*S*)-3,6-diaminohexanoyl]amino}methyl)-12-(hydroxymethyl)-2,5,8,11-14-pentaoxo-1,4,7,10,13-pentaazacyclohexadecan-6-ylidene}methyl]urea.

Capreomycin IB: sulfate salt of [(*Z*){(3*S*,9*S*,12*S*,15*S*)-15-amino-3-[(4*R*)-2-amino-1,4,5,6-tetrahydropyrimidin-4-yl]-9-({[(3*S*)-3,6-diaminohexanoyl]amino}methyl)-12-methyl-2,5,8,11-14-pentaoxo-1,4,7,10,13-pentaazacyclohexadecan-6-ylidene}methyl]urea.

Capreomycin IIA: sulfate salt of [(*Z*){(3*S*,9*S*,12*S*,15*S*)-15-amino-9-(aminomethyl)-3-[(4*R*)-2-amino-1,4,5,6-tetrahydropyrimidin-4-yl]-12-(hydroxymethyl)-2,5,8,11-14-pentaoxo-1,4,7,10,13-pentaazacyclohexadecan-6-ylidene}methyl]urea.

Capreomycin IIB: sulfate salt of [(*Z*){(3*S*,9*S*,12*S*,15*S*)-15-amino-9-(aminomethyl)-3-[(4*R*)-2-amino-1,4,5,6-tetrahydropyrimidin-4-yl]-12-methyl-2,5,8,11-14-pentaoxo-1,4,7,10,13-pentaazacyclohexadecan-6-ylidene}methyl]urea.

CAS Reg. no. 1405-37-4 (for capreomycin sulfate).

Description. A white or almost white powder.

Solubility. Very soluble in water, practically insoluble in ethanol (~750 g/L) TS and in ether.

Category. Antituberculosis drug.

Storage. Capreomycin sulfate should be kept in a tightly closed container or, if sterile, in a hermetically closed container.

Labelling. The label states, where applicable:

(1) that the substance is free from bacterial endotoxins;

(2) that the substance is sterile.

Requirements

Definition. Capreomycin sulfate is a mixture of the sulfates of antimicrobial polypeptides produced by the growth of *Streptomyces capreolus*. It contains not less than 70.0% of capreomycin, calculated with reference to the dried substance and taking into account the sum of capreomycin IA, IB, IIA and IIB. The content of capreomycin IA and IB is not less than 90.0% of the sum of capreomycin IA, IB, IIA and IIB.

Identity tests

-Either tests A, B and F or tests C, D, E and F may be applied.

A. Carry out the examination as described under *1.7 Spectrophotometry in the infrared region*. The infrared absorption spectrum is concordant with the spectrum obtained from capreomycin sulfate for identification RS or with the reference spectrum of capreomycin sulfate.

B. Carry out the test as described under *1.14.1 Chromatography*, Thin-layer chromatography using silica gel R5 as the coating substance and a mixture of 30 volumes of phenol R, 10 volumes of water R and 1 volume of ammonia (~260 g/L) TS as the mobile phase. Apply separately to the plate 4 µL of each of the following two solutions in water R. For solution (A) use 10 mg of the test substance per mL and for solution (B) use 10 mg of capreomycin sulfate for identification RS per mL. After removing the plate from the chromatographic chamber allow it to dry exhaustively in air. Spray with triketohydrindene/methanol TS and heat the plate for 3 minutes at 120 °C. Examine the chromatogram in daylight.

The spots obtained with solution A correspond in position, appearance and intensity with those obtained with solution B.

C. Carry out the test as described under *1.14.4 High performance liquid chromatography* using the conditions and solutions given under “Assay”. The retention times of the four major peaks in the chromatogram obtained with solution (1) correspond to the retention times of the peaks due to capreomycin IA, IB, IIA and IIB in the chromatogram obtained with solution (2).

D. The absorption spectrum (*1.6*) of a 20 µg/mL solution of the test substance in hydrochloric acid (0.1 mol/L) VS, when observed between 230 nm and 350 nm, exhibits a maximum at about 268 nm.

E. The absorption spectrum (*1.6*) of a 20 µg/mL solution of the test substance in sodium hydroxide (0.1 mol/L) VS, when observed between 230 nm and 350 nm, exhibits a maximum at about 287 nm.

F. A 20 mg/mL solution of the test substance yields reaction A described under *2.1 General identification tests* as characteristic of sulfates.

pH value (*1.3*). pH of a 30 mg/mL solution of the test substance in carbon-dioxide-free water R, 4.5–7.5.

Loss on drying. Dry for 4 hours at 100°C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 100 mg/g.

Heavy metals. Use 1.0 g of the test substance for the preparation of the test solution as described under *2.2.3 Limit test for heavy metals*, Procedure 3; determine the heavy metals content according to Method A; not more than 30 µg/g.

Sulfated ash (*2.3*). Not more than 10.0 mg/g.

Bacterial endotoxins. If intended for use in the manufacture of a parenteral dosage form, carry out the test as described under *3.4 Test for bacterial endotoxins*; contains not more than 0.5 IU of endotoxin per mg of capreomycin sulfate.

Sterility. If intended for use in the manufacture of either a parenteral or other sterile dosage form without a further appropriate sterilization procedure, complies with *3.2 Test for sterility*.

Related substances. Carry out the test as described under *1.14.4 High performance liquid chromatography* using the conditions given under “Assay”.

Prepare the following solutions using Mobile phase A as diluent. For solution (1) use 2.0 mg of the test substance per ml. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration of 10 µg of capreomycin sulfate per mL.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 268 nm.

Inject 20 µL of solution (1). The test is not valid unless the resolution between the two major peaks corresponding to capreomycin IA (with a relative retention of about 0.89) and capreomycin IB (retention time about 38 minutes) is at least 2.0 and the resolution between the peaks corresponding to capreomycin IIA and capreomycin IIB (with a relative retention of 0.53 and 0.63, respectively) is at least 3.5.

Inject separately 20 µL each of solutions (1) and (2).

In the chromatogram obtained with solution (1):

-the area of any peak, other than the four major peaks corresponding to capreomycin IA, IB, IIA and IIB, is not greater than 4

times the sum of the areas of the four major peaks obtained with solution (2) (2.0%);

- the area of not more than one such peak is greater than twice the sum of the areas of the four major peaks obtained with solution (2) (1.0%);
- the sum of the areas of all peaks, other than the four major peaks, is not greater than 14 times the sum of the areas of the four major peaks obtained with solution (2) (7.0%). Disregard any peak with an area less than 0.1 times the sum of the areas of the four major peaks in the chromatogram obtained with solution (2) (0.05%).

Assay. Carry out the test as described under *1.14.4 High performance liquid chromatography* using a stainless steel column (25 cm x 4.6 mm) packed with base deactivated particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 µm).

The mobile phases for the gradient elution consist of a mixture of mobile phase A and mobile phase B, using the following conditions:

mobile phase A: 5 volumes of acetonitrile R and 95 volumes of phosphate buffer pH 2.3;

mobile phase B: 15 volumes of acetonitrile R and 85 volumes of phosphate buffer pH 2.3.

Prepare the phosphate buffer pH 2.3 by dissolving 54.4 g of potassium dihydrogen phosphate R in 1500 mL of water R, adjust the pH to 2.3 by adding phosphoric acid (~105 g/L) TS, add 9.4 g of sodium hexanesulfonate R and dilute to 2000 mL with water R.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0–25	55–52	45–48	Linear gradient
25–40	52	48	Isocratic
40–60	30	70	Isocratic
60–70	55	45	Isocratic re-equilibration

Prepare the following solutions using mobile phase A as diluent. For solution (1) use 2.0 mg of the test substance per mL. For solution (2) dissolve the content of a vial of capreomycin sulfate RS in 5.0 mL.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 268 nm.

Inject 20 µL of solution (1). The assay is not valid unless the resolution between the two major peaks corresponding to capreomycin IA (with a relative retention of 0.89) and capreomycin IB (retention time about 38 minutes) is at least 2.0 and the resolution between the peaks corresponding to capreomycin IIA and capreomycin IIB (with a relative retention of 0.53 and 0.63, respectively) is at least 3.5.

Inject separately 20 µL each of solutions (1) and (2).

Measure the areas of the peak responses for capreomycin IA, IB, IIA and IIB obtained in the chromatograms from solutions (1) and (2) and, using the sum of the areas, calculate the percentage content of capreomycin using the declared content in capreomycin sulfate RS.