



**CAPREOMYCIN SULFATE**  
**DRAFT PROPOSAL FOR**  
***THE INTERNATIONAL PHARMACOPOEIA***  
**(AUGUST 2010)**  
***DRAFT FOR COMMENT***

This document was provided by a quality control expert and was discussed at the recent WHO consultation on specifications for medicines and quality control laboratory issues. Previous comments received have been incorporated into this revised draft. Should you have any comments, please send these to Dr S. Kopp, Manager, Medicines Quality Assurance Programme, Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland; e-mail: [kopps@who.int](mailto:kopps@who.int) with a copy to Ms C. Mendy [mendyc@who.int](mailto:mendyc@who.int) by 1 October 2010.

**During the past few years we have moved more towards an electronic system for sending out our draft monographs for comment, for convenience and in order to speed up the process. If you do not already receive our documents electronically, please let us have your e-mail address (to [bonnyw@who.int](mailto:bonnyw@who.int)) and we will add it to our electronic mailing list.**

---

© World Health Organization 2010

All rights reserved.

This draft is intended for a restricted audience only, i.e. the individuals and organizations having received this draft. The draft may not be reviewed, abstracted, quoted, reproduced, transmitted, distributed, translated or adapted, in part or in whole, in any form or by any means outside these individuals and organizations (including the organizations' concerned staff and member organizations) without the permission of WHO. The draft should not be displayed on any web site.

Please send any request for permission to:

Dr Sabine Kopp, Quality Assurance Programme, Medicines Quality Assurance Programme, Quality & Safety: Medicines (QSM), Department of Essential Medicines and Pharmaceutical Policies (EMP), World Health Organization, CH-1211 Geneva 27, Switzerland. E-mail: [kopps@who.int](mailto:kopps@who.int).

The designations employed and the presentation of the material in this draft do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

All reasonable precautions have been taken by the World Health Organization to verify the information contained in this draft. However, the printed material is being distributed without warranty of any kind, either expressed or implied. The responsibility for the interpretation and use of the material lies with the reader. In no event shall the World Health Organization be liable for damages arising from its use.

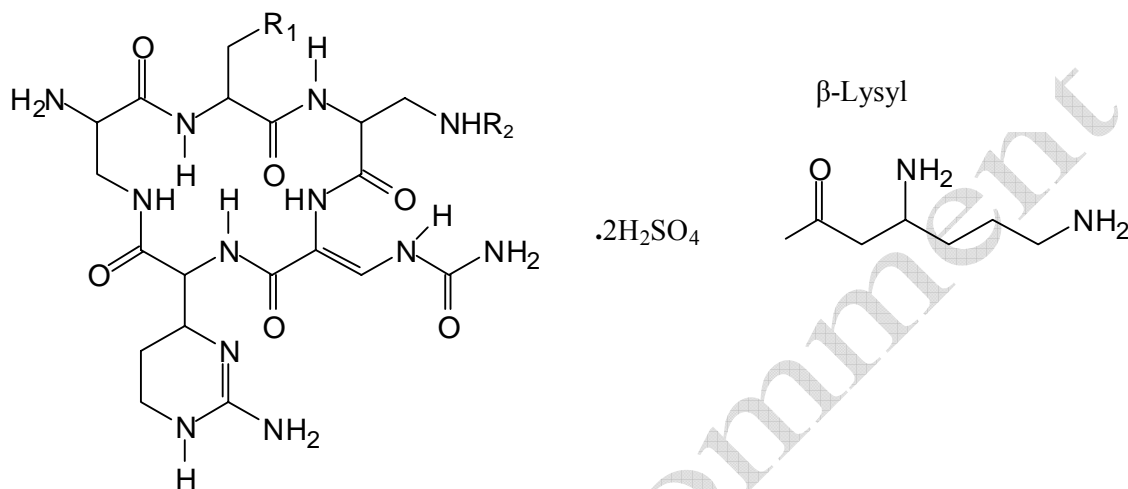
This draft does not necessarily represent the decisions or the stated policy of the World Health Organization.

**SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT**  
**QAS/10.357**  
*International Pharmacopoeia monograph on Capreomycin sulfate*

	<b>Date</b>
Preparation of first draft by laboratory	February 2010
Discussion of first draft in telephone conference	25 March 2010
Discussion of the draft monograph in the consultation on specifications for medicines and quality control laboratory issues	10-12 May 2010
Mailing of draft monograph for comments	August 2010
Collation of comments received	September-October 2010
Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations	18-22 October 2010
Any further action as required	...

**Draft proposal for *The International Pharmacopoeia*  
(August 2010)**

**CAPREOMYCINI SULFAS  
CAPREOMYCIN SULFATE**



Component	R <sub>1</sub>	R <sub>2</sub>
Capreomycin IA	OH	β-Lysyl
Capreomycin IB	H	β-Lysyl
Capreomycin IIA	OH	H
Capreomycin IIB	H	H

Capreomycin IA: C<sub>25</sub>H<sub>48</sub>N<sub>14</sub>O<sub>16</sub>S<sub>2</sub>; Capreomycin IB: C<sub>25</sub>H<sub>48</sub>N<sub>14</sub>O<sub>15</sub>S<sub>2</sub>; Capreomycin IIA: C<sub>19</sub>H<sub>35</sub>N<sub>12</sub>O<sub>15</sub>S<sub>2</sub>; Capreomycin IIB: C<sub>19</sub>H<sub>35</sub>N<sub>12</sub>O<sub>14</sub>S<sub>2</sub>

**Relative molecular mass.** Capreomycin IA: 864.9; Capreomycin IB: 848.9, Capreomycin IIA: 735.7; Capreomycin IIB: 719.7

**Chemical name.** Capreomycin IA: (3*S*)-3,6-diamino-*N*-[[[(2*S*,5*S*,8*Z*,11*S*,15*S*)-15-amino-11-[(4*R*)-2-amino-3,4,5,6-tetrahydropyrimidin-4-yl]-8-[(carbamoylamino)methylidene]-2-(hydroxymethyl)-3,6,9,12,16-pentaoxo-1,4,7,10,13-pentazacyclohexadec-5-yl]methyl]hexanamide; sulfuric acid.

Capreomycin IB: (3*S*)-3,6-diamino-*N*-[[[(2*S*,5*S*,8*Z*,11*S*,15*S*)-15-amino-11-[(4*R*)-2-amino-3,4,5,6-tetrahydropyrimidin-4-yl]-8-[(carbamoylamino)methylidene]-2-methyl-3,6,9,12,16-pentaoxo-1,4,7,10,13-pentazacyclohexadec-5-yl]methyl]hexanamide; sulfuric acid.

Capreomycin IIA: [(Z)-[(3*S*,9*S*,12*S*,15*S*)-15-amino-9-(aminomethyl)-3-[(4*R*)-2-amino-3,4,5,6-tetrahydropyrimidin-4-yl]-12-(hydroxymethyl)-2,5,8,11,14-pentaoxo-1,4,7,10,13-pentazacyclohexadec-6-ylidene]methyl]urea; sulfuric acid.

Capreomycin IIB: [(Z)-[(3*S*,9*S*,12*S*,15*S*)-15-amino-9-(aminomethyl)-3-[(4*R*)-2-amino-3,4,5,6-tetrahydropyrimidin-4-yl]-12-methyl-2,5,8,11,14-pentaoxo-1,4,7,10,13-pentazacyclohexadec-6-ylidene]methyl]urea; sulfuric acid.

CAS Reg. No. 1405-37-4 (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 the disulfate salt of capreomycin, a polypeptide mixture produced by the growth of *Streptomyces capreolus*. It contains not less than 93.0% and not more than 102.0% of capreomycin sulfate, calculated with reference to the dried substance and taking into account the sum of capreomycins sulfate IA, IB, IIA and IIB. The content of capreomycins sulfate IA and IB is not less than 90%.

## Identity tests

- Either tests A and E or tests B, C, D and E 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 RS or with the *reference spectrum* of capreomycin sulfate.
  - B. Carry out the test as described under 1.14.1 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 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. The absorption spectrum of a 20 µg/ml solution in hydrochloric acid (0.1 mol/l) VS, when observed between 230 nm and 350 nm, exhibits one maximum at about 268 nm; the specific absorbance ( $A_{1\text{cm}}^{1\%}$ ) is about 300.
- D. The absorption spectrum of a 20 µg/ml in sodium hydroxide (0.1 mol/l) VS, when observed between 230 nm and 350 nm, exhibits a major maximum at about 287 nm; the specific absorbance ( $A_{1\text{cm}}^{1\%}$ ) is about 200.
- E. A 20 mg/ml solution 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 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 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 0.35 IU of endotoxin per mg of capreomycin.

**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 3.2.2 Sterility testing of antibiotics, applying the membrane filtration test procedure and using the sampling plan described under 3.2.1 Test for sterility of non-injectable preparations.

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

Prepare the following solutions using water R 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 and capreomycin IB, with a relative retention of 0.89 and 1, respectively, is at least 2.0. The test is also not valid unless 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 capreomycins 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

- Either method A or method B may be applied.

A. 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)<sup>1</sup>.

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 to 52	45 to 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 water R as diluent. For solution (1) use 2.0 mg of the test substance per ml. For solution (2) use 2.0 mg of capreomycin sulfate RS per ml.

<sup>1</sup> Hypersil BDS column has been found suitable.

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  $\mu$ l of solution (1). The assay is not valid unless the resolution between the two major peaks corresponding to capreomycin IA and capreomycin IB, with a relative retention of 0.89 and 1, respectively, is at least 2.0. The assay is also not valid unless 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  $\mu$ l each of solutions (1) and (2).

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2) and calculate the content of capreomycin sulfate (sum of the four peaks corresponding to capreomycins IA, IB, IIA and IIB) from the declared content of capreomycin sulfate in capreomycin sulfate RS.

B. Dissolve about 40 mg, accurately weighed, in hydrochloric acid (0.1 mol/l) VS to produce 20 ml. Dilute 1 ml of this solution to 100 ml with the same solvent. Measure the absorbance of this solution in a 1-cm layer at the maximum at about 268 nm, and calculate the content of capreomycin sulfate, using the absorptivity value of 30.0 ( $A_{1\text{cm}}^{1\%} = 300$ ).

\*\*\*