Proposed revision of the monograph on
Capreomycin sulfate (Capreomycini sulfas)
for The International Pharmacopoeia

(May 2017)

DRAFT FOR COMMENT

Should you have any comments on this draft, please send these to Dr Herbert Schmidt, Medicines Quality Assurance Programme, Technologies Standards and Norms, Department of Essential Medicines and Health Products, World Health Organization, 1211 Geneva 27, Switzerland; fax: (+41 22) 791 4730 or email: schmidt@who.int by 31 July 2017.

In order to speed up the process for receiving draft monographs and for sending comments, please let us have your email address (to bonnyw@who.int) and we will add it to our electronic mailing list. Please specify if you wish to receive monographs.

© World Health Organization 2017

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 the World Health Organization. The draft should not be displayed on any website.

Please send any request for permission to:
Dr Sabine Kopp, Group Lead, Medicines Quality Assurance Programme, Technologies Standards and Norms, Department of Essential Medicines and Health Products, World Health Organization, CH-1211 Geneva 27, Switzerland. Fax: (41-22) 791 4730; email: 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/16.68:

Proposed revision of the monograph on

Capreomycin sulfate (Capreomycini sulfas)

<table>
<thead>
<tr>
<th>Date</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>November 2016</td>
<td>Revision drafted taking into consideration decisions made at the meeting of WHO Expert Committee on Specifications for Pharmaceutical Preparations in October 2016</td>
</tr>
<tr>
<td>2–4 May 2017</td>
<td>Discussion at consultation on new medicines, quality control and laboratory standards</td>
</tr>
<tr>
<td>June–August 2017</td>
<td>Draft revision sent out for public consultation</td>
</tr>
<tr>
<td>16–20 October 2017</td>
<td>Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations</td>
</tr>
<tr>
<td></td>
<td>Further follow-up action as required</td>
</tr>
</tbody>
</table>

[Note from the Secretariat. It is proposed to revise the monograph as follows:

- add a new reference substance – Capreomycin sulfate for identification RS – suitable for identity tests A and B (identification by IR and TLC);
- add a note of the Secretariat with respect to ongoing discussions about the transition from microbiological to physicochemical assays for antibiotics;
- update the style of the monograph.

Changes from the current monograph are indicated in the text by insert or delete.]
Proposed revision of the monograph on
Capreomycin sulfate (Capreomycini sulfas)

[Note from the Secretariat. The user of the monograph should note that the monograph
describes a chromatographic assay to determine if the concentrations of capreomycin IA, IB,
IIA and IIB of a sample under investigation complies with the definition (see section
definition). Other pharmacopoeias have the activity of the substance determined for assay by
means of microbiological methods. A correlation between the concentration of IA, IB, IIA
and IIB and the activity of the substance, determined with microbiological methods, has not
been established yet.]

<table>
<thead>
<tr>
<th>Component</th>
<th>R1</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capreomycin IA</td>
<td>OH</td>
<td>β-Lysyl</td>
</tr>
<tr>
<td>Capreomycin IB</td>
<td>H</td>
<td>β-Lysyl</td>
</tr>
<tr>
<td>Capreomycin IIA</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>Capreomycin IIB</td>
<td>H</td>
<td>H</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Capreomycin (base)</th>
<th>IA</th>
<th>IB</th>
<th>IIA</th>
<th>IIB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular formula</td>
<td>C_{25}H_{44}N_{14}O_{8}</td>
<td>C_{25}H_{44}N_{14}O_{7}</td>
<td>C_{19}H_{32}N_{12}O_{7}</td>
<td>C_{19}H_{32}N_{12}O_{6}</td>
</tr>
<tr>
<td>Relative molecular mass</td>
<td>668.7</td>
<td>652.7</td>
<td>540.5</td>
<td>524.5</td>
</tr>
<tr>
<td>CAS Reg. no.</td>
<td>37280-35-6</td>
<td>33490-33-4</td>
<td>62639-89-8</td>
<td>62639-90-1</td>
</tr>
<tr>
<td>Theoretical value of n in neutral sulfate salt</td>
<td>2</td>
<td>2</td>
<td>1.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Chemical names

Capreomycin IA: sulfate salt of ((Z)((3S,9S,12S,15S)-15-amino-3-[(4R)-2-amino-1,4,5,6-
tetrahydropyrimidin-4-yl]-9-(([(3S)-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){(3S,9S,12S,15S)-15-amino-3-[(4R)-2-amino-1,4,5,6-tetrahydropyrimidin-4-yl]-9-[(3S,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){(3S,9S,12S,15S)-15-amino-9-(aminomethyl)-3-[(4R)-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){(3S,9S,12S,15S)-15-amino-9-(aminomethyl)-3-[(4R)-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 contents 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 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 for identification 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 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. 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; the specific absorbance (A<sub>1</sub><sub>cm</sub><sup>1%</sup>) is about 300.

D. 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; the specific absorbance (A<sub>1</sub><sub>cm</sub><sup>1%</sup>) is about 200.

E. 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 0.350.5 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” — Method A.
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) with a relative retention of 0.89 and 1, respectively, is at least 2.0 and . 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 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 all peaks 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 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 hexanesulphonate R and dilute to 2000 mL with water R.
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) use 2.0 mg of capreomycin sulfate RS 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 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), with a relative retention of 0.89 and 1, respectively, is at least 2.0 and 3.5, respectively. 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 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 IA, IB, IIA and IIB using from the declared content in capreomycin sulfate RS.

***