CONCEPT PAPER FOR COMMENT

TRANSITION FROM MICROBIOLOGICAL TO PHYSICOCHEMICAL ASSAYS IN MONOGRAPHS ON CAPREOMYCIN ACTIVE PHARMACEUTICAL INGREDIENTS AND PRODUCTS

(April 2017)

DRAFT FOR COMMENTS

Should you have any comments on the attached text, please send these to Dr Herbert Schmidt, Medicines Quality Assurance, Technologies Standards and Norms, World Health Organization, 1211 Geneva 27, Switzerland; email: schmidt@who.int; fax: (+41 22) 791 4730 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.
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TRANSITION FROM MICROBIOLOGICAL TO PHYSICOCHEMICAL ASSAYS IN MONOGRAPHS ON CAPREOMYCIN ACTIVE PHARMACEUTICAL INGREDIENTS AND PRODUCTS

[Note from the Secretariat of The International Pharmacopoeia. The strength of antibiotics can be determined using microbiological or physicochemical assays. While traditionally microbiological methods were predominantly used in quality control of antibiotics, physicochemical methods are nowadays preferred for various reasons. The transition from microbiological to physicochemical assays has been largely completed for single-component antibiotics. For multicomponent antibiotics, however, the use of physicochemical methods remains challenging.

Following discussions and decisions at meetings of the WHO Expert Committees on Specifications for Pharmaceutical Preparations and on Biological Standardization, the Secretariat of The International Pharmacopoeia is seeking information and international collaboration in order to establish a chromatographic assay as an alternative to microbiological assays for the essential medicine capreomycin powder for injection and the corresponding active pharmaceutical ingredient (API) capreomycin sulfate. In addition, this initiative aims at harmonizing quality control requirements for these products. It may also provide new insights which can facilitate transitions of other antibiotics.

The Secretariat invites stakeholders, in particular regulatory authorities, pharmacopoeias and manufacturers of capreomycin sulfate, capreomycin powders for injection and other medicines containing multicomponent antibiotics, to comment on the proposals made in this document. Subsequent steps, in particular the performance of the bridging study to link the mass with the activity of capreomycin, will be decided inter alia based on the discussions of the comments received.]

Scope of the document

This document proposes steps to finish the transition of the tuberculostatic aminoglycoside capreomycin that has been started with the publication of chromatographic assay methods in the monographs on Capreomycin sulfate and Capreomycin powder for injection of The International Pharmacopoeia. In the course of the transition, factors that may pose a risk to the safety of patients shall be identified and controlled, in particular by means of two surveys: a landscape analysis of capreomycin APIs and products on the global market and a comparison of national capreomycin reference substances. Besides, this proposal aims at the international harmonization of quality control requirements for capreomycin.

Background information

Antibiotics produced by fermentation often consist of complex mixtures of structurally related components with different activities. Microbiological methods were historically used to quantify the total activity of these mixtures. As evidence of their structure and composition increased, transitions from microbiological to physicochemical assays, in particular chromatographic methods, were possible and envisaged as they are often more discriminative and easier or faster to perform.
Microbiological assays, on the other hand, measure the total (in vitro) activity of antibiotics against a reference microorganism, integrating all moieties that contribute to this effect.

While the transition from microbiological to physicochemical assays has been largely completed for single-component antibiotics, it remains challenging for substances containing several components.

Discussions at meetings of WHO Expert Committees

Points to consider when switching from biological to physicochemical assays were discussed at the meetings of the Expert Committee on Specifications for Pharmaceutical Preparations (ECSPP) and the Expert Committee on Biological Standardization (ECBS) in 2007. In 2009, the ECSPP recommended that microbiological assays shall be replaced by, in particular, chromatographic methods, where possible and appropriate. Following this decision, chromatographic assays were elaborated and published as part of the monographs on Capreomycin sulfate and Capreomycin for injection in *The International Pharmacopoeia*.

Following the publication of these monographs, the comparability of analytical results gained with the new chromatographic assay method and with so far used microbiological assays was discussed. At the meetings of the ECSPP and ECBS in 2016 it was agreed that the Secretariat of *The International Pharmacopoeia* should contact manufacturers to obtain further information about the prevailing composition of capreomycin active pharmaceutical ingredient (API), methods used to determine the content of capreomycin powders for injection and information regarding a correlation between the mass and the microbiological activity of the antibiotic.

Capreomycin for injection in the WHO Model List of Essential Medicines

In the WHO Model List of Essential Medicines (EML) (19th Edition) the strength of capreomycin powder for injection is given as “1 g (as sulfate) in vial”. Considering that in the past pharmacopoeias described microbiological methods for the assay of capreomycin products, the information regarding the strength given in the EML should be interpreted as “capreomycin sulfate equivalent to the activity of 1 g capreomycin in vial”. This interpretation would correspond to the way the comparator product, Capastat®, is labelled, namely “Each vial contains the equivalent of 1 g capreomycin activity”.

The monographs on Capreomycin sulfate and Capreomycin for injection in *The International Pharmacopoeia*

Capreomycin is a mixture of four structurally related compounds, Capreomycin IA, IB, IIA and IIB with different specific activities. In the monograph on Capreomycin sulfate the active substance is defined on a mass basis: “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”.

The Chinese Pharmacopoeia (CP), the Indian Pharmacopoeia (IP) and the United States Pharmacopeia (USP) have similar requirements regarding the composition of Capreomycin sulfate. However, in these pharmacopoeias the capreomycin content of APIs and finished products is determined using microbiological methods.
In 1969, the specific activities of the isolated four main components were determined. The results of these investigations showed that there is a significant difference between the activities of components IA versus IB and I versus II. As the applied techniques to separate and purify substances have become more specific and efficient in past decades, WHO was advised to re-establish the data should succeeding decisions be based on them.

While the monograph on Capreomycin currently limits the capreomycin II contents to maximum 10%, the ratio between capreomycin IA and IB is not defined at present. Further information and guidance is sought regarding the relevance of such an additional limit with a view to ensure that products even with extreme differences in the IA and IB concentrations consistently comply or not comply with the different compendial assays.

**Capreomycin sulfate reference substances**

Subsequent to the publication of the capreomycin monographs, a reference substance, capreomycin sulfate ICRS Batch 1, was established for use according to the prescribed compendial tests. Following a comprehensive analytical characterization of the candidate material, a defined capreomycin base concentration per vial, expressed in mass units, was assigned to the standard to render it suitable, i.e. for assay by high performance liquid chromatography (HPLC).

The ECSPP released capreomycin sulfate ICRS Batch 1 at its meeting in 2016 with the following note in the leaflet: “The International Chemical Reference Substance for capreomycin sulfate ICRS is intended to be used as described in The International Pharmacopoeia for assay by HPLC according to the monographs for capreomycin sulfate and capreomycin for injection. The substance is suitable to serve as a reference for the quantitative determination of the content of capreomycins IA, IB, IIA and IIB from the declared content in capreomycin sulfate RS. 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.”

A Capreomycin WHO International Standard for Antibiotics (ISA) to define the activity of capreomycin in microbiological assays was established in 19681 and discontinued in 2000 following an enquiry to determine whether there was a continued necessity for the standard.2 The reference substance served as a primary reference standard for pharmacopoeias to calibrate their national, secondary reference standards, subsequently used in routine laboratory tests and assays.

**Landscape analysis of capreomycin APIs and products on the global market**

The aim of this survey is to provide an overview on the composition of capreomycin APIs and products on the market. Together with information on the activity and toxicity of the different components, the results of the chromatographic analysis will help to evaluate the comparability of capreomycin products. Based on the results of this survey, additional limits regarding the chemical composition of capreomycin, in particular a limit to specify the ratio IA to IB, shall be discussed and implemented if need be.

To initiate the survey, WHO shall invite manufactures to share the following information and samples:

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Manufacturers of capreomycin or capreomycin sulfate:

1. A sample of capreomycin or capreomycin sulfate (about 10 g), representative for the authorized manufacturing process, together with the certificate of analysis and the material safety data sheet.
2. A compilation of the specifications of the product together with a description of the methods used to determine them. For the methods to determine the content/strength and composition of the product the reference substance(s) used, the name(s) of the authorizing organization(s) and the declared strength(s) or assigned content(s) shall be indicated. In case chromatographic methods are used sample chromatograms shall also be submitted.
3. The outcome of investigations to correlate the total microbiological activity of capreomycin/capreomycin sulfate (or the activity of the components) with the mass concentration of the components (including information about the design of the performed study, a description of the methods used and details of the results obtained) (if available).
4. Information about the toxicity of capreomycin with respect to its composition (if available).

Manufacturers of capreomycin powder for injection:

1. A sample of each authorized capreomycin powder for injection (10 vials each of 1 g for each product belonging to the same batch, together with the corresponding certificate(s) of analysis) and a copy of the packaging indicating the labelled strength of the products.
2. A compilation of the specifications of the product together with a description of the methods used to determine them. For the methods to determine the content/strength and composition of the product the reference substance(s) used, the name(s) of the authorizing organization(s) and the declared strength(s) or assigned content(s) shall be indicated. In case chromatographic methods are used sample chromatograms shall also be submitted.
3. The outcome of investigations to correlate the total microbiological activity of capreomycin/capreomycin sulfate (or the activity of the components) with the mass concentration of the components (including information about the design of the performed study, a description of the methods used and details of the results obtained) (if available).
4. Information about the toxicity of capreomycin with respect to its composition (if available).

Comparison of national capreomycin reference substances

Not only assay methods based on different principles, also the lack of an international primary reference substance defining the activity of capreomycin may have affected the comparability of capreomycin dose regimes over time. To obtain relevant evidence, WHO shall organize laboratory investigations to determine:

1. the antimicrobial activity of a common sample, capreomycin sulfate International Chemical Reference Substances (ICRS)³, according to the current provisions in the CP, IP and USP; and
2. the percentage mass concentrations of capreomycin IA, IB, IIA and IIB of the national reference substances prescribed by CP, IP and USP and analysed using the HPLC method described in the monograph on Capreomycin sulfate of The International Pharmacopoeia.

³ Capreomycin sulfate ICRS is proposed as a common test sample because the chemical composition of the substance was thoroughly investigated during its establishment as a reference substances for physico-chemical tests according to The International Pharmacopoeia. The available analytical data, together with the results of the antimicrobiological determination may help to understand and to establish the correlation between the composition of capreomycin and its activity. Capreomycin ICRS is also needed as a reference substances for the determination under (2).
Based on the results of this survey, WHO shall evaluate jointly with i.a. the concerned pharmacopoeias the need to re-establish capreomycin ISA. The results will also help to further elucidate how the composition of capreomycin determines its activity.

**Bridging study to link the mass with the activity of capreomycin**

Considering the results of the landscape analysis of capreomycin APIs and products and on the comparison of national capreomycin reference substances, pharmacopoeias (in particular the CP, IP, USP and *The International Pharmacopoeia*) may decide to finish the transition from microbiological to a physicochemical assay for the capreomycin content by performing a bridging study to link the mass with the activity of the substance. Such a linkage would allow manufacturers to retain the current labelling of their products (i.e. the strength labelled in activity) and to seek regulatory approval to use a chromatographic method for the testing of their products.

A USP guidance document provides points to consider for the development of chromatographic or other physicochemical methods to replace microbiological assays. As a pivotal step, the process would involve the separation and purification of each antimicrobial moiety, process impurity and degradation product of the antibiotic and a subsequent determination of their individual, relative microbial activity.

To determine these relative microbial activities an international (primary) reference substance, capreomycin ISA, which defines the activity of capreomycin sulfate, would have to be re-established.

The alternative chromatographic method should be composition- and stability-indicating and would have to consider the specific absorptivity of the different components (in case the absorptivities differ significantly). The already published HPLC method in *The International Pharmacopoeia* is proposed to be used for this purpose.

**International harmonization of pharmacopoeial requirements for capreomycin**

The joint bridging study and its results shall also foster harmonization of pharmacopoeial requirements for capreomycin API and products. With the knowledge of the correlation between the composition and activity of capreomycin other pharmacopoeias, in particular CP, IP and USP, may consider to also switch to the alternative chromatographic method published in *The International Pharmacopoeia*.

In addition, the gained insights may facilitate future transitions from microbiological to physicochemical assays in monographs of other multicomponent antibiotics.

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4 USP 39, chapter 1223, Validation of alternative methods to antibiotic microbial assays.