Consultation documents

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The International Pharmacopoeia

Mebendazole tablets
(Mebendazoli compressi)

This is a draft proposal of a monograph for The International Pharmacopoeia (Working document QAS/16.685, March 2017).

The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects. Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, 1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidth@who.int.

Category. Anthelmintic.

Storage. Mebendazole tablets should be kept in a tightly closed container.

Additional information. Strengths in the current WHO Model List of Essential Medicines (EML): 100 mg, 500 mg. Strengths in the current WHO EML for children: 100 mg, 500 mg.

Requirements

Comply with the monograph for Tablets.

Definition. Mebendazole tablets contain not less than 90.0% and not more than 110.0% of the amount of mebendazole (C₁₆H₁₃N₃O₃) stated on the label.

Manufacture. The formulation, manufacturing process and product packaging of mebendazole tablets are designed and controlled so as to minimize the conversion of the polymorphic form of mebendazole from C to A. They ensure that, at any stage of the life cycle of the product, when tested by a suitable method such as infrared spectrometry (see Identity test A) or X-ray powder diffractometry, the mebendazole in the tablets is predominantly in the form of polymorph C.
Identity tests

- Either tests A and B or tests A and C may be applied.

A. To a quantity of the powdered tablets containing 0.05 g of Mebendazole add 20 mL of water R, shake, filter and wash the residue with three quantities, each of 10 mL of water R. Dry the residue overnight under vacuum at room temperature and carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The two infrared absorption bands at about 3405 cm\(^{-1}\) and 1720 cm\(^{-1}\) are concordant with those in the spectrum obtained from mebendazole RS (containing mebendazole polymorph C).

B. Carry out the test as described under 1.14.1 Thin-layer chromatography using silica gel R6 as the coating substance and a mixture of 85 volumes of dichloromethane R, 5 volumes of methanol R, 5 volumes of acetone R and 5 volumes of anhydrous formic acid R as the mobile phase. Apply separately to the plate 5 μL of each of the following solutions. For solution (A) add 2 mL of formic acid R to a quantity of the powdered tablets containing 20 mg of mebendazole and sonicate for about 5 minutes. Add 18 mL of acetone R, mix, filter and use the filtrate. For solution (B) dissolve 10 mg of mebendazole RS in 1 mL of formic acid R and shake. Add 9 mL of acetone R and mix. After removing the plate from the chromatographic chamber allow it to dry in air and examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution (A) corresponds in position, appearance and intensity with that obtained with solution (B).

D. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions under “Assay”. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time of the peak due to mebendazole obtained with solution (2).

Related substances

Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (10 cm × 4.6 mm) packed with base-deactivated particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (3 μm).\(^1\)

Use the following conditions for gradient elution:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (%) v/v</th>
<th>Mobile phase B (%) v/v</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–15</td>
<td>80 to 70</td>
<td>20 to 30</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>15–20</td>
<td>70 to 10</td>
<td>30 to 90</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>20–25</td>
<td>10</td>
<td>90</td>
<td>Isocratic</td>
</tr>
<tr>
<td>25–26</td>
<td>10 to 80</td>
<td>90 to 20</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>26–36</td>
<td>80</td>
<td>20</td>
<td>Isocratic re-equilibration</td>
</tr>
</tbody>
</table>

Operate with a flow rate of 1.2 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 250 nm. Maintain the column temperature at 40°C.

\(^1\) A HYPERSIL BDS C\(_{18}\) column has been found suitable.
Prepare as a solvent a mixture of 60 volumes of methanol R and 40 volumes of water R.

For solution (1) transfer a quantity of the powdered tablets, containing about 100 mg of mebendazole, accurately weighed, to a 100 mL volumetric flask. Add 30 mL of anhydrous formic acid R and sonicate for about 20 minutes. Dilute to volume with solvent mixture, mix and filter. For solution (2) dilute 1.0 mL of solution (1) to 100.0 mL with the solvent mixture. Dilute 5.0 mL of this solution to 20.0 mL with the solvent mixture. For solution (3) transfer 10 mg mebendazole RS to a 10 mL volumetric flask, add 5 mL of methanol R and 1 mL of sodium hydroxide (−40 g/L) TS solution, heat in a water bath at 60°C for 1 hour, cool to room temperature and adjust the solution to pH 7 with hydrochloric acid (−36.5 g/L) TS. Dilute with methanol R to volume and mix.

Inject 10 µL of solution (3). Use the chromatogram to identify the peak due to impurity A. The impurity is eluted at the relative retention of 0.4 with reference to mebendazole (retention time about 12 minutes).

The test is not valid unless in the chromatogram obtained with solution (3) the resolution between mebendazole and impurity A is at least 10.

Inject alternately 10 µL each of solution (1) and (2).

In the chromatogram obtained with solution (1):

• the area of any peak corresponding to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.25%).

**Dissolution**

For 100 mg tablets. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms using 900 mL of hydrochloric acid (−3.65 g/L) TS as the dissolution medium and rotating the paddle at 75 revolutions per minute. At 120 minutes withdraw a sample of 10 mL of the dissolution medium through an in-line filter. Allow the filtered sample to cool to room temperature. Dilute 5.0 mL of the filtrate to 50.0 mL with the dissolution medium.

Determine the content of mebendazole (C\textsubscript{16}H\textsubscript{13}N\textsubscript{3}O\textsubscript{3}) in the medium by 1.14.4 High-performance liquid chromatography using the conditions described under "Assay" and a suitable solution of mebendazole RS as a reference solution.

For each of the six tablets tested calculate the total amount of mebendazole (C\textsubscript{16}H\textsubscript{13}N\textsubscript{3}O\textsubscript{3}) in the medium using the declared content of C\textsubscript{16}H\textsubscript{13}N\textsubscript{3}O\textsubscript{3} in mebendazole RS. The amount in solution for each tablet is not less than 60% (Q) of the amount declared on the label.

For 500 mg tablets. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms using 900 mL of a 1.0% solution of sodium dodecyl sulfate R in hydrochloric acid (−0.365 g/L) TS as the dissolution medium and rotating the paddle at 75 revolutions per minute. At 60 minutes withdraw a sample of 10 mL of the dissolution medium through an in-line filter. Allow the filtered sample to cool to room temperature. Dilute 1.0 mL of the filtrate to 50.0 mL with the dissolution medium.
Determine the content of mebendazole (C\textsubscript{16}H\textsubscript{13}N\textsubscript{3}O\textsubscript{3}) in the medium by 1.14.4 High-performance liquid chromatography using the conditions described under “Assay” and a suitable solution of mebendazole RS as a reference solution.

For each of the six tablets tested calculate the total amount of mebendazole (C\textsubscript{16}H\textsubscript{13}N\textsubscript{3}O\textsubscript{3}) in the medium using the declared content of C\textsubscript{16}H\textsubscript{13}N\textsubscript{3}O\textsubscript{3} in mebendazole RS. The amount in solution for each tablet is not less than 70% (Q) of the amount declared on the label.

**Assay**

Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (10 cm × 4.6 mm) packed with octadecylsilyl base-deactivated silica gel for chromatography R (3 µm).

As the mobile phase use a solution prepared as follows: dissolve 7.5 g of ammonium acetate R in 1000 mL of water R, mix and filter. Mix 750 mL of this solution with 250 mL of acetonitrile R.

Prepare as a solvent a mixture of 60 volumes of methanol R and 40 volumes of water R.

Prepare the following solutions. For solution (1) weigh and powder 20 tablets. Transfer a quantity of the powdered tablets, containing about 100 mg of mebendazole, accurately weighed, to a 100 mL volumetric flask. Add 30 mL of anhydrous formic acid and sonicate for about 20 minutes. Dilute to volume with solvent mixture, mix and filter. Dilute 5.0 mL of the filtrate to 100.0 mL with the solvent mixture. For solution (2) transfer 25.0 mg of mebendazole RS to a 25 mL volumetric flask, add 10 mL of the anhydrous formic acid R and sonicate to dissolve. Dilute to volume with the solvent mixture. Dilute 5.0 mL of this solution to 100.0 mL with the solvent mixture.

Operate with a flow rate of 1.2 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 250 nm.

Inject alternately 10 µL each of solutions (1) and (2).

Measure the areas of the peaks corresponding to mebendazole obtained in the chromatograms from solution (1) and (2) and calculate the percentage content of mebendazole (C\textsubscript{16}H\textsubscript{13}N\textsubscript{3}O\textsubscript{3}) in the tablets using the declared content of C\textsubscript{16}H\textsubscript{13}N\textsubscript{3}O\textsubscript{3} in mebendazole RS.

**Reagents to be established**

Hydrochloric acid (~0.365 g/L) TS

Hydrochloric acid (~250 g/L) TS, dilute with water to contain 0.365 g of HCl in 1000 mL.

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2  A HYPERSIL BDS C18 column has been found suitable.
Proposed revision of the monograph on

Capreomycin sulfate
(Capreomycini sulfas)

This is a draft revision of a monograph for The International Pharmacopoeia (Working document QAS/16.689, May 2017). 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.

The working document with line numbers for commenting is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects. In the online document changes from the current monograph are indicated in the text by insert or delete.

[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>
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<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>
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<tr>
<td>Relative molecular mass</td>
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<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\text{-amino-3-}\{(4R)-2\text{-amino-1,4,5,6-tetrahydropyrimidin-4-yl}\}-9-\{(3S)-3,6\text{-diamino-hexanoyl}\text{amino}\}\text{methyl}\}-12\text{-}(\text{hydroxymethyl})-2,5,8,11-14\text{-penta-oxo}-1,4,7,10,13\text{-penta-aza-}\text{cyclohexadecan-6-ylidene}\}\text{methyl}\}\text{urea}.

Capreomycin IB: sulfate salt of \(((Z)\{(3S,9S,12S,15S)-15\text{-amino-3-}\{(4R)-2\text{-amino-1,4,5,6-tetrahydropyrimidin-4-yl}\}-9-\{(3S)-3,6\text{-diamino-hexanoyl}\text{amino}\}\text{methyl}\}-12\text{-methyl-2,5,8,11-14-penta-oxo}-1,4,7,10,13\text{-penta-aza-}\text{cyclohexadecan-6-ylidene}\}\text{methyl}\}\text{urea}.

Capreomycin IIA: sulfate salt of \(((Z)\{(3S,9S,12S,15S)-15\text{-amino-9-(aminomethyl)-3-}\{(4R)-2\text{-amino-1,4,5,6-tetrahydropyrimidin-4-yl}\}-12\text{-}(\text{hydroxymethyl})-2,5,8,11-14\text{-penta-oxo}-1,4,7,10,13\text{-penta-aza-}\text{cyclohexadecan-6-ylidene}\}\text{methyl}\}\text{urea}.

Capreomycin IIB: sulfate salt of \(((Z)\{(3S,9S,12S,15S)-15\text{-amino-9-(aminomethyl)-3-}\{(4R)-2\text{-amino-1,4,5,6-tetrahydropyrimidin-4-yl}\}-12\text{-methyl-2,5,8,11-14-penta-oxo}-1,4,7,10,13\text{-penta-aza-}\text{cyclohexadecan-6-ylidene}\}\text{methyl}\}\text{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 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.

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.

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 0.5 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.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–25</td>
<td>55–52</td>
<td>45–48</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>25–40</td>
<td>52</td>
<td>48</td>
<td>Isocratic</td>
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<tr>
<td>40–60</td>
<td>30</td>
<td>70</td>
<td>Isocratic</td>
</tr>
<tr>
<td>60–70</td>
<td>55</td>
<td>45</td>
<td>Isocratic re-equilibration</td>
</tr>
</tbody>
</table>

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) 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.

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Proposed revision of the monograph on

Capreomycin for injection
(Capreomycini ad injectionem)

This is a draft revision of a monograph for The International Pharmacopoeia (Working document QAS/16.690, May 2017). It is proposed to revise the monograph as follows:

- add a new reference substance - Capreomycin sulfate for identification RS - suitable for identity test 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;
- determine the percentage content of capreomycin per sealed container;
- update the style of the monograph.

The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects. In the online document changes from the current monograph are indicated in the text by insert or delete.

[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.]

Description. A white or almost white powder.

Category. Antituberculosis drug.

Storage. Capreomycin for injection should be stored in a well-closed container.

Labelling. The designation on the container of capreomycin for injection should state that the active ingredient is in the sulfate form and the quantity should be indicated in terms of the equivalent amount of capreomycin.

Additional information. Strength in the current WHO Model List of Essential Medicines (EML): 1 g. Strength in the current EML for children: 1 g.

The injection is reconstituted by dilution of Capreomycin powder for injections in Water for injections.

The reconstituted injection should be used immediately after preparation.
Requirements

The powder for injection and the reconstituted injection comply with the monograph for Parenteral preparations.

Definition. Capreomycin for injection is a sterile powder containing Capreomycin sulfate. It contains not less than 90.0% and not more than 115.0% of the amount of capreomycin stated on the label, taking into account 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) dissolve a quantity of the powder to obtain a solution containing 10 mg of the powder for injection per mL. 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. Dissolve a quantity of the powder for injection in hydrochloric acid (0.1 mol/L) VS to obtain a solution containing the equivalent of 20 μg of capreomycin per mL. The absorption spectrum (1.6) of this solution, when observed between 230 nm and 350 nm, exhibits a maximum at about 268 nm.

D. Dissolve a quantity of the powder for injection in sodium hydroxide (0.1 mol/L) VS to obtain a solution containing the equivalent of 20 μg of capreomycin per mL. The absorption spectrum (1.6) of this solution, when observed between 230 nm and 350 nm, exhibits a maximum at about 287 nm.

E. A solution of the powder for injection containing the equivalent of 20 mg of capreomycin per mL yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Clarity of solution. A freshly prepared solution of the powder for injection containing the equivalent of 1 g of capreomycin in 10 mL of carbon-dioxide-free water R is clear.

pH value (1.13). pH of a solution of the powder for injection containing the equivalent of 0.3 g of capreomycin in 10 mL of carbon-dioxide-free water R, 4.5–7.5.

Bacterial endotoxins. 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.
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) dissolve a quantity of the powder for injection to obtain a solution containing the equivalent of 2.0 mg of capreomycin per mL. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration of 10 µg of capreomycin 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.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–25</td>
<td>55–52</td>
<td>45–48</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>25–40</td>
<td>52</td>
<td>48</td>
<td>Isocratic</td>
</tr>
<tr>
<td>40–60</td>
<td>30</td>
<td>70</td>
<td>Isocratic</td>
</tr>
<tr>
<td>60–70</td>
<td>55</td>
<td>45</td>
<td>Isocratic re-equilibration</td>
</tr>
</tbody>
</table>
Weigh and mix the contents of 5 containers. Prepare the following solutions using mobile phase A as diluent. For solution (1) dissolve a quantity of the mixed contents, containing the equivalent of about 100 mg of capreomycin, accurately weighed, and dilute to 50.0 mL. For solution (2) use a solution containing 2.75 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) 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 per sealed container using the declared content in capreomycin sulfate RS.

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Concept paper for comment

Transition from microbiological to physicochemical assays in monographs on capreomycin active pharmaceutical ingredients and products

This is a concept paper (Working document QAS/16.695, April 2017) proposed by 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 of The International Pharmacopoeia 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 a bridging study to link the mass with the activity of capreomycin, will be decided inter alia based on the discussions of the comments received.

The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects.

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”.

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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 1968\(^1\) 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:

**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

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\(^1\) WHO Technical Report Series, No. 384.

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 antimicrobiological 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.

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\(^4\) provides points to consider for the development of chromatographic or other physicochemical methods to replace microbiological assays. As a

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\(^3\) 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).

\(^4\) USP 39, chapter 1223, Validation of alternative methods to antibiotic microbial assays.
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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Proposed revision of the monograph on

Atenolol
(Atenololum)

This is a draft revision of a monograph for The International Pharmacopoeia (Working document QAS/17.700, May 2017). It is proposed to revise the monograph based on information found in the European Pharmacopoeia and in the scientific literature.

The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects. In the online document changes from the current monograph are indicated in the text by insert or delete.

![Chemical structure of Atenolol](image)

C₁₄H₂₂N₂O₃

**Relative molecular mass.** 266.3

**Chemical name.** 2-[(p-[2-Hydroxy-3-(isopropylamino)propoxy]phenyl)acetamide (racemate); CAS Reg. No. 29122-68-7.

**Description.** A white or almost white powder.

**Solubility.** Sparingly soluble in water; soluble in ethanol (~750 g/L) TS; slightly soluble in dichloromethane R.

**Category.** Cardiovascular agent; β-adrenoreceptor blocking agent.

**Storage.** Atenolol should be kept in a tightly closed container.

**Requirements**

Atenolol contains not less than 99.0% and not more than 101.0% of C₁₄H₂₂N₂O₃, calculated with reference to the dried substance.

**Identity tests**

- Either test A or tests B and C 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 atenolol RS or with the reference spectrum of atenolol.
B. The absorption spectrum of a 0.10 mg/mL solution in methanol R, when observed between 230 nm and 350 nm, exhibits 2 maxima at about 275 nm and 282 nm. The ratio of the absorbance at 275 nm to that at 282 nm is between 1.15 and 1.20.

C. Carry out the test as described under 1.14.1 Thin-layer chromatography using silica gel R4 as the coating substance and a mixture of 99 volumes of methanol R and 1 volume of ammonia (~260 g/L) TS as the mobile phase. Apply separately to the plate 10 μL of each of 2 solutions in methanol R containing (A) 10 mg of the test substance per mL and (B) 10 mg of atenolol RS per mL. After removing the plate from the chromatographic chamber allow it to dry in air and examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution (A) corresponds in position, appearance and intensity with that obtained with solution (B).

**Solution S.** Dissolve 0.10 g of the test substance in carbon-dioxide-free water R and dilute to 10.0 mL with the same solvent.

**Optical rotation** (1.4). Use solution S; α = +0.10 to –0.10.

**Clarity and colour of solution.** Solution (S) is clear and not more intensely coloured than degree 6 of the range of reference solutions of the most appropriate colour, when compared as described under 1.11.2 Degree of coloration of liquids, Method II.

*Note from the Secretariat. Chapter 1.11 Colour of liquids is currently under revision. Reference is already made to a new test procedure to be added under section 1.11.2 Degree of coloration of liquids.*

**Chlorides.** Dissolve 0.25 g in a mixture of 2 mL of nitric acid (~130 g/L) TS and 20 mL of water and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 1.0 mg/g.

**Sulfated ash** (2.3). Not more than 1.0 mg/g, determined using 1.0 g.

**Loss on drying.** Dry 1.0 g of the test substance to constant mass at 105°C; it loses not more than 5.0 mg/g.

**Related substances.** Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (12.5 cm × 4.0 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 μm). Prepare the following solution to be used as the mobile phase: dissolve 1.0 g of sodium octanesulfonate R and 0.4 g of tetrabutylammonium hydrogen sulfate R in 1000 mL of a mixture of 80 volumes of a 3.4 mg/mL solution of potassium dihydrogen phosphate R, the pH of the solution adjusted to 3.0 with phosphoric acid (~1440 g/L), 18 volumes of methanol R and 2 volumes of tetrahydrofuran R.

Prepare the following solutions in mobile phase. For solution (1) dissolve 50 mg of the test substance in 20 mL and dilute to 25.0 mL. For solution (2) dilute 1.0 mL of solution (1) to 100.0 mL. Dilute 1.0 mL of this solution to 10.0 mL. For solution (3) dissolve 2 mg of atenolol for system suitability RS (containing atenolol and the impurities B, F, G, I and J) in 1.0 mL of the mobile phase.

Operate with a flow rate of 0.6 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 226 nm.
Inject 10 μL of solution (3). Record the chromatograms for about 5 times the retention time of atenolol (retention time about 8 minutes). Use the chromatogram obtained with solution (3) and the chromatogram supplied with atenolol for system suitability RS to identify the peaks due to atenolol and the impurities B, F, G, I and J.

The test is not valid unless the resolution between the peaks due to the impurities J and I is at least 1.4.

Inject alternately 10 μL each of solutions (1) and (2).

In the chromatogram obtained with solution (1):

• the area of any peak corresponding to impurity B is not greater than 2 times the area of the peak due to atenolol in the chromatogram obtained with solution (2) (0.2%);
• the area of any peak corresponding to either impurity F, G, I or J is not greater than 1.5 times the area of the peak due to atenolol in the chromatogram obtained with solution (2) (0.15%);
• the area of any other impurity peak is not greater than the area of the peak due to atenolol in the chromatogram obtained with solution (2) (0.10%);
• the sum of the areas of all impurity peaks is not greater than 5 times the area of the peak due to atenolol in the chromatogram obtained with solution (2) (0.5%). Disregard any peak with an area less than 0.5 times the area of the peak due to atenolol in the chromatogram obtained with solution (2) (0.05%).

Assay. Dissolve about 0.200 g, accurately weighed, in 80 mL of glacial acetic acid R1 and titrate with perchloric acid (0.1 mol/L) VS as described under 2.6 Non-aqueous titration, Method A, determining the end-point potentiometrically.

Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 26.63 mg of C_{14}H_{22}N_{2}O_{3}.

Impurities

A. 2-(4-hydroxyphenyl)acetamide

B. 2-[4-[(2RS)-2,3-dihydroxypropoxy]phenyl]acetamide

D. 2-[4-[(2RS)-3-chloro-2-hydroxypropoxy]phenyl]acetamide
E. 2,2’-[2-hydroxypropane-1,3-diyl]bis(oxy-4,1-phenylene)]diacetamide

F. 2,2’-[[propan-2-yl]azanediyl]bis[(2-hydroxypropane-3,1-diyl)oxy-4,1-phenylene)]diacetamide


I. 2-[4-[(2RS)-3-(ethylamino)-2-hydroxypropoxy]phenyl]acetamide

J. 2-[4-[(2RS)-3-amino-2-hydroxypropoxy]phenyl]acetamide
1.15.1

Capillary electrophoresis

This is a draft proposed text for The International Pharmacopoeia (Working document QAS/16.698, May 2017). This text is based on the internationally harmonized texts developed by the Pharmacopoeial Discussion Group (PDG). It has been developed in line with the style and requirements used in The International Pharmacopoeia. The permission to reproduce the text will be requested when the text is adopted by the WHO Expert Committee on Specifications for Pharmaceutical Preparations.

The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects.

This text is based on the internationally harmonized texts developed by the Pharmacopoeial Discussion Group (PDG). It has been developed in line with the style and requirements used in The International Pharmacopoeia.

General principles

Capillary electrophoresis is a physical method of analysis based on the migration, inside a capillary, of charged analytes dissolved in an electrolyte solution under the influence of a direct-current electric field.

The migration velocity of the analyte under an electric field of intensity \( E \) is determined by the electrophoretic mobility of the analyte and the electroosmotic mobility of the buffer inside the capillary. The electrophoretic mobility of a solute (\( \mu_{ep} \)) depends on the characteristics of the solute (electrical charge, molecular size and shape) and the characteristics of the buffer in which the migration takes place (type and ionic strength of the electrolyte, pH, viscosity and additives). The electrophoretic velocity (\( v_{ep} \)) of a solute, assuming a spherical shape, is as follows:

\[
 v_{ep} = \frac{q}{6\pi \eta r} \times \left( \frac{V}{L} \right)
\]

in which \( q \) is the effective charge of the solute; \( \eta \) is the viscosity of the electrolyte solution; \( r \) is the Stoke's radius of the solute; \( V \) is the applied voltage; and \( L \) is the total length of the capillary.

When an electric field is applied through the capillary filled with buffer, a flow of solvent, called electroosmotic flow, is generated inside the capillary. Its velocity depends on the electroosmotic mobility (\( \mu_{eo} \)), which in turn depends on the charge density on the capillary internal wall and the buffer characteristics. The electroosmotic velocity (\( v_{eo} \)) is given by the equation:

\[
 v_{eo} = \frac{\varepsilon \zeta}{\eta} \times \left( \frac{V}{L} \right)
\]

in which \( \varepsilon \) is the dielectric constant of the buffer; \( \zeta \) is the zeta potential of the capillary surface; and the other terms are as defined above.
The velocity of the solute ($v$) is given by the equation:

$$v = v_{ep} + v_{eo}$$

The electrophoretic mobility of the analyte and the electroosmotic mobility may act in the same direction or in opposite directions, depending on the charge of the solute. In normal capillary electrophoresis, anions will migrate in the opposite direction to the electroosmotic flow and their velocities will be smaller than the electroosmotic velocity. Cations will migrate in the same direction as the electroosmotic flow and their velocities will be greater than the electroosmotic velocity. Under conditions in which there is a fast electroosmotic velocity with respect to the electrophoretic velocity of the solutes, both cations and anions can be separated in the same run.

The time ($t$) taken by the solute to migrate the distance ($l$) from the injection end of the capillary to the detection point (capillary effective length) is as follows:

$$t = \frac{l}{v_{ep} + v_{eo} = \frac{l}{(\mu_{ep} + \mu_{eo}) \times V}}$$

in which the other terms are as defined above.

In general, uncoated fused-silica capillaries above pH 3 have negative charge due to ionized silanol groups in the inner wall. Consequently, the electroosmotic flow is from anode to cathode. The electroosmotic flow must remain constant from run to run to obtain good reproducibility in the migration velocity of the solutes. For some applications, it might be necessary to reduce or suppress the electroosmotic flow by modifying the inner wall of the capillary or by changing the concentration, composition and/or the pH of the buffer solution.

After the introduction of the sample into the capillary each analyte ion of the sample migrates within the background electrolyte as an independent zone according to its electrophoretic mobility. Zone dispersion, that is the spreading of each solute band, results from different phenomena. Under ideal conditions, the sole contribution to the solute-zone broadening is molecular diffusion of the solute along the capillary (longitudinal diffusion). In this ideal case, the efficiency of the zone, expressed as the number of theoretical plates ($N$), is given by:

$$N = \frac{(\mu_{ep} + \mu_{eo}) \times V \times l}{2 \times D \times L}$$

in which $D$ is the molecular diffusion coefficient of the solute in the buffer.

In practice, other phenomena, such as heat dissipation, sample adsorption onto the capillary wall, mismatched conductivity between sample and buffer, length of the injection plug, detector cell size and unlevelled buffer reservoirs, can also significantly contribute to band dispersion. Separation between two bands (expressed by the resolution $R_s$) can be obtained by modification of the electrophoretic mobility of the analytes, by the electroosmotic mobility induced in the capillary and by increasing the efficiency for the band of each analyte as follows:

$$R_s = \frac{\sqrt{N} (\mu_{epb} - \mu_{epa})}{4 (\mu_{ep} + \mu_{eo})}$$

in which $\mu_{epa}$ and $\mu_{epb}$ are the electrophoretic mobilities of the two analytes to be separated; is the average electrophoretic mobility of the two analytes calculated as:

$$\bar{\mu}_{ep} = \frac{1}{2} (\mu_{epb} + \mu_{epa})$$
Apparatus
An apparatus for capillary electrophoresis is composed of a high voltage controllable direct current power supply; two buffer reservoirs held at the same level and containing specified anodic and cathodic solutions; two electrode assemblies (cathode and anode) immersed in the buffer reservoirs and connected to the power supply; a separation capillary usually made of fused-silica, sometimes with an optical viewing window aligned with the detector, depending on the detector type, with the ends of the capillary placed in the buffer reservoirs and the capillary being filled with a solution specified in a given monograph; a suitable injection system; a detector capable of monitoring the amount of substance of interest passing through a segment of the separation capillary at a given time, generally based on absorption spectrophotometry (ultraviolet (UV) and visible), fluorimetry, conductimetric, amperometric, or mass spectrometric detection, depending on the specific applications, or even indirect detection to detect non-UV-absorbing and nonfluorescent compounds; a thermostatic system capable of maintaining a constant temperature inside the capillary, recommended to obtain good separation reproducibility; a recorder; and a suitable integrator or a computer.

The definition of the injection process and its automation are critical for precise quantitative analysis. Modes of injection include gravity, pressure or vacuum, or electrokinetic injection. The amount of each sample component introduced electrokinetically depends on its electrophoretic mobility, leading to possible discrimination using this injection mode.

It is expected that the capillary, the buffer solutions, the preconditioning method, the sample solution, and the migration conditions will be specified in the individual monograph. The electrolytic solution employed is filtered to remove particles and degassed to avoid bubble formation that could interfere with the detection system or interrupt the electrical contact in the capillary during the separation run. To achieve reproducible migration time of the solutes, it would be necessary to develop, for each analytical method, a rigorous rinsing routine.

Capillary zone electrophoresis

Principle
In capillary zone electrophoresis, analytes are separated in a capillary containing only buffer without any anticonvective medium. In this technique, separation takes place because the different components of the sample migrate as discrete bands with different velocities. The velocity of each band depends on the electrophoretic mobility of the solute and the electroosmotic flow on the capillary (see “General principles”). Coated capillaries can be used to increase the separation capacity of those substances adsorbing on fused-silica surfaces.

This mode of capillary electrophoresis is appropriate for the analysis of small (molecular weight < 2000) and large (2000 < MW < 100,000) molecules. Due to the high efficiency achieved in capillary zone electrophoresis, separation of molecules having only minute differences in their charge-to-mass ratio can be effected. This separation mode also allows the separation of chiral compounds by addition of chiral selectors to the separation buffer.
**Optimization**

Optimization of the separation is a complex process where several separation parameters can play a major role. The main factors to be considered in the development of the separations are instrumental and electrolytic solution parameters.

**Instrumental parameters**

**Voltage.** A Joule heating plot is useful in optimizing the applied voltage and column temperature. The separation time is inversely proportional to applied voltage. However, an increase in the voltage used can cause excessive heat production, giving rise to temperature and, as a result, viscosity gradients in the buffer inside the capillary, which causes band broadening and decreases resolution.

**Polarity.** Electrode polarity can be normal (anode at the inlet and cathode at the outlet) and the electroosmotic flow will move toward the cathode. If the electrode polarity is reversed the electroosmotic flow is away from the outlet and only charged analytes with electroosmotic mobilities greater than the electroosmotic flow will pass to the outlet.

**Temperature.** The main effect of temperature is observed on buffer viscosity and electrical conductivity, thus affecting migration velocity. In some cases, an increase in capillary temperature can cause a conformational change of some proteins, modifying their migration time and the efficiency of the separation.

**Capillary.** The length and internal diameter of the capillary affects the analysis time, the efficiency of separations and the load capacity. Increasing both effective length and total length can decrease the electric fields, at a constant voltage, which increases migration time. For a given buffer and electric field, heat dissipation (thus, sample band broadening) depends on the internal diameter of the capillary. The latter also affects the detection limit, depending on the sample volume injected into the capillary and the detection system used.

The adsorption of sample components on the capillary wall limits efficiency; therefore, methods to avoid these interactions should be considered in the development of a separation method. In the specific case of proteins, several strategies have been devised to avoid adsorption on the capillary wall. Some of these strategies (use of extreme pH and adsorption of positively charged buffer additives) only require modification of the buffer composition to prevent protein adsorption. Other strategies include the coating of the internal wall of the capillary with a polymer covalently bonded to the silica that prevents interaction between the proteins and the negatively charged silica surface. For this purpose, ready-to-use capillaries with coatings consisting of neutral-hydrophilic, cationic and anionic polymers are commercially available.

**Electrolytic solution parameters**

**Buffer type and concentrations.** Suitable buffers for capillary electrophoresis have an appropriate buffer capacity in the pH range of choice and low mobility to minimize current generation.

To minimize band distortion, it is important to match buffer-ion mobility to solute mobility whenever possible. The type of sample solvent used is important to achieve on-column sample focusing, which increases separation efficiency and improves detection. Also, an increase in buffer concentration at a given pH decreases electroosmotic flow and solute velocity.
**Buffer pH.** The pH of the buffer can affect separation by modifying the charge of the analyte or additives and by changing the electroosmotic flow. For protein and peptide separation, a change in the pH of the buffer from above the isoelectric point (pI) to below the pI changes the net charge of the solute from negative to positive. An increase in the buffer pH generally increases the electroosmotic flow.

**Organic solvents.** Organic modifiers, such as methanol, acetonitrile and others may be added to the aqueous buffer to increase the solubility of the solute or other additives and/or to affect the ionization degree of the sample components. The addition of these organic modifiers to the buffer generally causes a decrease in the electroosmotic flow.

**Additives for chiral separations.** To separate optical isomers, a chiral selector is added to the separation buffer. The most commonly used chiral selectors are cyclodextrins, although in some cases crown ethers, certain polysaccharides or even proteins can be used. Because chiral recognition is governed by the different interactions between the chiral selector and each of the enantiomers the resolution achieved for the chiral compounds depends largely on the type of chiral selector used.

While developing a given separation it may be useful to test cyclodextrins having a different cavity size (α-, β-, or γ-cyclodextrin) or modified cyclodextrins with neutral (methyl, ethyl, hydroxyalkyl, etc.) or ionizable (aminomethyl, carboxymethyl, sulfobutylether, etc.) moieties. When using modified cyclodextrins, batch-to-batch variations in the degree of substitution of the cyclodextrins must be taken into account because it will influence the selectivity. The resolution of chiral separations is also controlled by the concentration of the chiral selector, the composition and pH of the buffer and the separation temperature. Organic additives, such as methanol or urea, can also affect the resolution of separation.

**Capillary gel electrophoresis**

In capillary gel electrophoresis, separation takes place inside a capillary filled with a gel that acts as a molecular sieve. Molecules with similar charge-to-mass ratios are separated according to molecular size because smaller molecules move more freely through the network of the gel and therefore migrate faster than larger molecules. Different biological macromolecules (for example, proteins and DNA fragments), which often have similar charge-to-mass ratios, can thus be separated according to their molecular mass by capillary gel electrophoresis.

**Characteristics of gels**

Two types of gels are used in capillary electrophoresis: permanently coated gels and dynamically coated gels. Permanently coated gels are prepared inside the capillary by polymerization of monomers. One example of such a gel is a cross-linked polyacrylamide. This type of gel is usually bonded to the fused-silica wall and cannot be removed without destroying the capillary. For protein analysis under reducing conditions the separation buffer usually contains sodium dodecyl sulfate and the sample is denatured by heating in a mixture of sodium dodecyl sulfate and 2-mercaptoethanol or dithiothreitol before injection. When non-reducing conditions are used (for example, analysis of an intact antibody), 2-mercaptoethanol and dithiothreitol are not used. Optimization of separation in a cross-linked gel is obtained by modifying the separation buffer (see “Capillary zone electrophoresis”) and by controlling the gel porosity during the gel preparation. For cross-linked polyacrylamide gels the porosity can
be modified by changing the concentration of acrylamide and/or the ratio of the cross-linker. As a rule, a decrease in the porosity of the gel leads to a decrease in the mobility of the solutes. Due to the rigidity of this type of gel, only electrokinetic injection can be used.

Dynamically coated gels are hydrophilic polymers (i.e. linear polyacrylamide, cellulose derivatives, dextran, etc.) which can be dissolved in aqueous separation buffers, giving rise to a separation medium that also acts as a molecular sieve. These polymeric separation media are easier to prepare than cross-linked polymers. They can be prepared in a vial and filled by pressure in a wall-coated capillary with no electroosmotic flow. Replacing the gel before every injection generally improves the separation reproducibility. The porosity of the dynamically coated gels can be increased by using polymers of higher molecular mass (at a given polymer concentration) or by decreasing the polymer concentration (for a given polymer molecular mass). A decrease in gel porosity leads to a decrease in the mobility of the solute for the same buffer. Both hydrodynamic and electrokinetic injection techniques can be used because the dissolution of these polymers in the buffer gives low viscosity solutions.

**Capillary isoelectric focusing**

**Principle**

In isoelectric focusing the molecules migrate under the influence of the electric field, so long as they are charged, in a pH gradient generated by ampholytes having pI values in a wide range (polyaminocarboxylic acids), dissolved in the separation buffer.

The three basic steps in capillary isoelectric focusing are loading, focusing and mobilization.

**Loading step.** Two methods may be employed.

Loading in one step: The sample is mixed with ampholytes and introduced into the capillary by pressure or vacuum.

Sequential loading: A leading buffer, then the ampholytes, then the sample mixed with ampholytes, again ampholytes alone, and finally the terminating buffer are introduced into the capillary. The volume of the sample must be small enough so as not to modify the pH gradient.

**Focusing step.** When the voltage is applied, ampholytes migrate toward the cathode or the anode according to their net charge, creating the pH gradient from anode (lower pH) to cathode (higher pH). During this step the components to be separated migrate until they reach a pH corresponding to their isoelectric point, and the current drops to very low values.

**Mobilization step.** If mobilization is required for detection, use one of the following three methods.

Method 1: Mobilization is accomplished during the focusing step, under the influence of the electroosmotic flow when this flow is small enough to allow the focusing of the components.

Method 2: Mobilization is accomplished by application of positive pressure after the focusing step.

Method 3: Mobilization is achieved after the focusing step by adding salts to the cathode reservoir or the anode reservoir, depending on the direction chosen for mobilization, in order to alter the pH in the capillary when the voltage is applied.
As the pH is changed the proteins and ampholytes are mobilized in the direction of the reservoir, which contains added salts and pass the detector. The separation achieved is expressed as $\Delta pI$ and depends on the pH gradient ($dpH/dx$), the number of ampholytes having different $pI$ values, the molecular diffusion coefficient ($D$), the intensity of the electric field ($E$) and the variation of the electrophoretic mobility of the analyte with the pH ($-d\mu/dpH$):

$$\Delta pI = 3 \times \sqrt{\frac{D(dpH/dx)}{E(-d\mu/dpH)}}$$

**Optimization**

The major parameters that need to be considered in the development of separations are the following:

**Voltage.** The use of high fields from 300 V/cm to 1000 V/cm during the focusing step.

**Capillary.** The electroosmotic flow must be reduced or suppressed depending on the mobilization strategy selected (see above). Coated capillaries tend to reduce the electroosmotic flow.

**Solutions.** The anode buffer reservoir is filled with a solution of a lower pH than the $pI$ of the most acidic ampholyte, and the cathode reservoir is filled with a solution with a higher pH than the $pI$ of the most basic ampholyte. Phosphoric acid for the anode and sodium hydroxide for the cathode are frequently used. Addition of a polymer, like methylcellulose, in the ampholyte solution tends to suppress convective forces (if any) and electroosmotic flow by increasing the viscosity. Commercial ampholytes covering many pH ranges are available and may also be mixed to obtain an expanded pH range. Broad pH ranges are used to estimate the $pI$, whereas narrower ranges are employed to improve accuracy. Calibration can be made by correlating migration time with the $pI$ of a series of standard protein markers. During the focusing step, precipitation of proteins at their $pI$ can be prevented, if necessary, using buffer additives such as glycerol, surfactants, urea, or zwitterionic buffers. However, depending on the concentration, urea can denature proteins.

**Micellar electrokinetic chromatography**

**Principle**

Separation takes place in an electrolytic solution that contains a surfactant at a concentration above the critical micellar concentration (CMC). The solute molecules are distributed between the aqueous buffer and the pseudostationary phase composed by the micelles according to the solute's partition coefficient. The technique can be considered as a hybrid of electrophoresis and chromatography. It is a technique that can be used for the separation of both neutral and charged solutes maintaining the efficiency, speed and instrumental suitability of capillary electrophoresis. One of the most widely used surfactants in micellar electrokinetic chromatography (MEKC) is the anionic surfactant, sodium dodecyl sulfate, although other surfactants, such as cationic surfactant cetyl trimethyl ammonium salts, have also been used.
The separation mechanism is as follows. At neutral and alkaline pH, a strong electroosmotic flow is generated and moves the separation buffer ions in the direction of the cathode. If sodium dodecyl sulfate is used as surfactant the electrophoretic migration of the anionic micelle is in the opposite direction, towards the anode. As a result, the overall micelle migration velocity is slowed compared to the bulk flow of the electrolytic solution. In the case of neutral solutes, because the analyte can partition between the micelle and the aqueous buffer and has no electrophoretic mobility, the analyte migration velocity will depend only on the partition coefficient between the micelle and the aqueous buffer. In the electropherogram the peaks corresponding to each uncharged solute are always between that of the electroosmotic flow marker and that of the micelle; and the time elapsed between these two peaks is called the separation window. For electrically charged solutes the migration velocity depends on both the partition coefficient of the solute between the micelle and the aqueous buffer and on the electrophoretic mobility of the solute in the absence of micelles.

Since the mechanism in MEKC of neutral and weakly ionized solutes is essentially chromatographic, migration of the solute and resolution can be rationalized in terms of the retention factor of the solute ($k'$), also referred to as mass distribution ratio ($D_m$), which is the ratio between the number of moles of solute in the micelle to those in the mobile phase. For a neutral compound, $k'$ is given as follows:

$$k' = \frac{t_r - t_0}{t_0 \times \left(1 - \frac{t_r}{t_{mc}}\right)} = k \times \frac{V_s}{V_m}$$

in which $t_r$ is the migration time of the solute; $t_0$ is the analysis time of the unretained solute obtained by injecting an electroosmotic flow marker that does not enter the micelle (e.g. methanol); $t_{mc}$ is the micelle migration time measured by injecting a micelle marker, such as Sudan III, which migrates continuously associated in the micelle; $K$ is the partition coefficient of the solute; $V_s$ is the volume of the micellar phase; and $V_m$ is the volume of the mobile phase.

The resolution between two closely-migrating solutes ($R_s$) is as follows:

$$R_s = \frac{\sqrt{N}}{4} \times \frac{\alpha - 1}{\alpha} \times \frac{k_{b'}'}{k_{a'}'} \times \frac{1 - \left(\frac{t_0}{t_{mc}}\right)}{1 + k_{a'} \times \left(\frac{t_0}{t_{mc}}\right)}$$

in which $N$ is the number of theoretical plates for one of the solutes; $\alpha$ is the selectivity; and $k_{a'}'$ and $k_{b'}'$ are retention factors for both solutes, respectively ($k_{b'}' > k_{a'}'$).

Similar, but not identical, equations give $k'$ and $R_s$ values for electrically charged solutes.

**Optimization**

The main parameters to be considered in the development of separations by MEKC are instrumental and electrolytic solution parameters.

**Instrumental parameters**

**Voltage.** Separation time is inversely proportional to applied voltage. However, an increase in voltage can cause excessive heat production that gives rise to temperature gradients and viscosity gradients of the buffer in the cross section of the capillary. This effect can be significant with high conductivity buffers, such as those containing micelles. Poor heat dissipation causes band broadening and decreases resolution.
**Temperature.** Variations in capillary temperature affect the partition coefficient of the solute between the buffer and the micelles, the critical micellar concentration and the viscosity of the buffer. These parameters contribute to the migration time of the solutes. The use of a good cooling system improves the reproducibility of the migration time for the solutes.

**Capillary.** As in capillary zone electrophoresis, length and internal diameter of the capillary contribute to analysis time and efficiency of separations. Increasing both effective length and total length can decrease the electrical fields, working at constant voltage, and will increase migration time and improve the separation efficiency. The internal diameter controls heat dissipation, for a given buffer and electrical field, and consequently broadening of the sample band.

**Electrolytic solution parameters**

**Surfactant type and concentration.** The type of surfactant, as the stationary phase in chromatography, affects the resolution because it modifies separation selectively. The log k′ of a neutral compound increases linearly with the concentration of surfactant in the mobile phase. When k′ approaches the value of

$$
\sqrt{\frac{t_{mc}}{t_0}}
$$

resolution in MEKC reaches a maximum. Modifying the concentration of surfactant in the mobile phase changes the resolution.

**Buffer pH.** pH does not modify the partition coefficient of non-ionized solutes, but it can modify the electroosmotic flow in uncoated capillaries. A decrease in the buffer pH decreases the electroosmotic flow and, therefore, increases the resolution of the neutral solutes in MEKC, resulting in a longer analysis time.

**Organic solvents.** To improve MEKC separation of hydrophobic compounds, organic modifiers (methanol, propanol, acetonitrile, etc.) can be added to the electrolytic solution. The addition of these modifiers generally decreases migration time and selectivity of the separation. The addition of organic modifiers affects critical micellar concentration; thus, a given surfactant concentration can be used only with a certain percentage of organic modifier before the micellization is inhibited or adversely affected, resulting in the absence of micelles and, therefore, the absence of the partition. The dissociation of micelles in the presence of a high content of organic solvent does not always mean that the separation will no longer be possible, because in some cases, the hydrophobic interaction between the ionic surfactant monomer and the neutral solutes forms solvophobic complexes that can be separated electrophoretically.

**Additives for chiral separations.** For the separation of enantiomers using MEKC a chiral selector is included in the micellar system, either covalently bound to the surfactant or added to the micellar separation electrolyte. Micelles that have a moiety with chiral discrimination properties include salts, N-dodecanoyl-l-amino acids, bile salts, etc. Chiral resolution can also be achieved using chiral discriminators, such as cyclodextrins, added to the electrolytic solutions that contain micellized achiral surfactants.
Other additives. Selectivity can be modified by adding chemicals to the buffer. Addition of several types of cyclodextrins to the buffer is also used to reduce the interaction of hydrophobic solutes with the micelle, increasing the selectivity for this type of compound. The addition of substances able to modify solute-micelle interactions by adsorption on the latter has been used to improve the selectivity of the separations in MEKC. These additives may consist of a second surfactant (ionic or nonionic), which gives rise to mixed micelles or metallic cations that dissolve in the micelle and form coordination complexes with the solutes.

Quantification

Peak areas must be divided by the corresponding migration time to give the corrected area in order to compensate for the shift in migration time from run to run, thus reducing the variation of the response. Dividing the peak areas by migration time will also compensate for the different responses of sample constituents with different migration times. Where an internal standard is used, check that no peak of the substance to be examined is masked by that of the internal standard.

Calculations

From the values obtained, calculate the content of a component or components being determined. When indicated, the percentage of one (or more) components of the sample to be examined is calculated by determining the corrected area(s) of the peak(s) as a percentage of the total of the corrected areas of all the peaks, excluding those due to solvents or any added reagents (normalization procedure). The use of an automatic integration system (integrator or data acquisition and processing system) is recommended.

System suitability

In order to check the behaviour of the capillary electrophoresis system, system suitability parameters are used. The choice of these parameters depends on the mode of capillary electrophoresis used. The parameters include the following: retention factor $k'$ (used only for micellar electrokinetic chromatography), apparent number of theoretical plates ($N$), the symmetry factor ($A_s$), and the resolution ($R_s$). In previous sections the theoretical expressions for $N$ and $R_s$ have been described but more practical equations that allow for the determination of these suitability parameters using the electropherograms are given below.

Apparent number of theoretical plates

The apparent number of $N$ may be calculated from the formula:

$$N = 5.54 \left( \frac{t_R}{w_h} \right)^2$$

in which $t_R$ is the migration time or distance along the baseline between the point of injection and the perpendicular dropped from the maximum of the peak corresponding to the component; and $w_h$ is the peak width at half-height.
**Resolution**

The R\textsubscript{s} between peaks of similar heights of two components may be calculated from the formula:

\[ R_s = 1.18 \frac{(t_{R2} - t_{R1})}{(w_{h1} + w_{h2})} \]

\[ t_{R2} > t_{R1} \]

in which \( t_{R1} \) and \( t_{R2} \) are the migration times or distances along the baseline between the point of injection and the perpendiculars dropped from the maxima of two adjacent peaks; and \( w_{h1} \) and \( w_{h2} \) are the peak widths at half-height.

When appropriate the R\textsubscript{s} may also be calculated by measuring the height of the valley (\( H_v \)) between two partly resolved peaks in a standard preparation, the height of the smaller peak (\( H_p \)), and calculating the peak-to-valley ratio:

\[ p/v = H_p/H_v \]

**Symmetry factor**

The symmetry factor of \( A_s \) may be calculated using the formula:

\[ A_s = \frac{w_{0.05}}{2d} \]

in which \( w_{0.05} \) is the width of the peak at one-twentieth of the peak height; and \( d \) is the distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at one-twentieth of the peak height.

Other suitability parameters include tests for area repeatability (standard deviation of areas or of area/migration time) and tests for migration time repeatability (standard deviation of migration time). Migration time repeatability provides a test for the suitability of the capillary washing procedures. An alternative practice to avoid the lack of repeatability of the migration time is to use a migration time relative to an internal standard.

**Signal-to-noise ratio**

A test for the verification of the signal-to-noise ratio for a standard preparation or the determination of the limit of quantification may also be useful for the determination of related substances. The detection limit and quantification limit correspond to a signal-to-noise ratio of 3 and 10, respectively. The signal-to-noise ratio (\( S/N \)) is calculated as follows:

\[ S/N = 2H/h \]

in which \( H \) is the height of the peak corresponding to the component concerned in the electropherogram obtained with the specified reference solution, measured from the maximum of the peak to the extrapolated baseline of the signal observed over a distance equal to twenty times the width at half-height; and \( h \) is the range of the background in an electropherogram obtained after injection of a blank, observed over a distance equal to twenty times the width at the half-height of the peak in the electropherogram obtained with the prescribed reference solution and, if possible, situated equally around the place where this peak would be found.
WHO Medicines quality guidelines

The following medicines quality-related guidelines have been posted for public comment on the WHO website. The respective working documents with line numbers are available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects.

- **Good practices for desk assessment**
  Guidance on good practices for desk assessment for compliance with good manufacturing practices, good laboratory practices and good clinical practices for marketing authorization of medical products
  Working document QAS/17.713 (May 2017)
  Inspection of manufacturing, testing, clinical trial and distribution sites poses an increasing burden on regulatory authorities. It is therefore good practice to rely on inspection information from other trusted authorities as part of risk-based inspection planning, so that there is no on-site inspection without well-founded cause. This text aims to provide general guidance on performing desk assessments in lieu of onsite inspections.

- **Considerations for requesting analysis of medicines samples**

- **Model certificate of analysis**
  These two documents are revisions of 2002 guidance texts. The proposed updates take into account new trends and international developments.

- **“SRA” collaborative procedure**
  Collaborative procedure in the assessment and accelerated national registration of pharmaceutical products approved by stringent regulatory authorities
  Working document QAS/17.704 (March 2017)
  This text proposes scheme for national medicines regulatory authorities and pharmaceutical companies (manufacturers) to facilitate registrations of medicines approved by stringent regulatory authorities.

- **Good herbal processing practices**
  Revised Draft: WHO guidelines on good herbal processing practices (GHPP) for herbal medicines
  WHO/SDS/TCM (March 2017)
  This text proposes technical guidance on processing of herbs to produce herbal materials, of herbal materials to produce herbal preparations, and of herbal materials or herbal preparations to produce herbal dosage forms.