Consultation documents

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The International Pharmacopoeia

Chlorhexidine digluconate solution
(Chlorhexidini digluconatis solutio)

This is a draft proposal for The International Pharmacopoeia (Working document QAS/16.668, June 2016). The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/.

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.

\[
C_{22}H_{30}Cl_{10}N_{10}O_{7}
\]

Relative molecular mass. 897.8

Chemical name. 1,1’-(hexane-1,6-diyl)bis[5-(4-chlorophenyl)biguanide] di-D-gluconate, 2,4,11,13-tetraazatetradecanediimidamide,N,N’-bis(4-chlorophenyl)-3,12-diimino-,di-D-gluconate; CAS Reg. No. 18472-51-0.

Description. Chlorhexidine digluconate is an aqueous solution of chlorhexidine digluconate. It is a clear, colourless or pale yellow liquid.

Miscibility. Miscible with water, with not more than 3 parts of acetone R and with not more than 5 parts of ethanol (~750 g/L) TS.

Category. Antiseptic.

Storage. Chlorhexidine digluconate should be kept in a well-closed container (avoid unlined steel containers), protected from light.
Requirements

Chlorhexidine digluconate solution contains not less than 190 g per L and not more than 210 g per L of \( C_{22}H_{30}Cl_2N_{10.2}C_6H_{12}O_7 \).

Identity tests

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

A. To 1 mL of the solution add 40 mL of water R, cool in iced water, make alkaline to titan yellow paper R by adding dropwise, and with stirring, sodium hydroxide (~420 g/L) TS and add 1 mL in excess. Filter, wash the precipitate with water R until the washings are free from alkali. Recrystallize from methanol R. Dry the crystals at 100–105°C. 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 chlorhexidine RS or with the reference spectrum of chlorhexidine. If the spectra thus obtained are not concordant, repeat the test using the residues obtained by separately dissolving the test substance and chlorhexidine RS in a small amount of methanol R and evaporating to dryness. The infrared absorption spectrum is concordant with the spectrum obtained from chlorhexidine RS.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography using silica R5 as the coating substance and a mixture of 10 volumes of ammonia (~260 g/L) TS, 10 volumes of ethyl acetate R, 30 volumes of water R and 50 volumes of ethanol (~750 g/L) TS as the mobile phase. Apply separately to the plate 5 μL of each of the following 2 solutions in water R. For solution (A) dilute 10 mL of the preparation to be examined to a final volume of 50 mL. For solution (B) use 25 mg of calcium gluconate R per mL. After removing the plate from the chromatographic chamber dry the plate at 100°C for 20 minutes and allow to cool. Spray the plate with a solution containing 25 g/L ammonium molybdate R and 10 g/L ceric sulfate R in sulfuric acid (~98 g/L) TS. Heat the plate for about 10 minutes at 110°C. Examine the chromatogram in daylight.

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

C. Dissolve 1 mL of the solution in 10 mL of water and add, with shaking, 0.15 mL of copper (II) chloride/ammonia TS; a purple precipitate is produced immediately. Continue to add 0.5 mL of copper(II)chloride/ammonia TS; the colour of the precipitate changes to blue.

D. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under “Related substances”. The retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (3).

pH value (1.13). Dilute 1 mL to 20 mL in carbon-dioxide-free water R, 5.5–7.0.

Relative density (1.3). \( d_{20}^{20} = 1.06 - 1.07 \).

Impurity P (p-Chloroaniline)

Prepare sample solution (A) by diluting 0.20 g of the test solution to 30 mL with water R. Add rapidly and with thorough mixing after each addition: 5 mL of hydrochloric acid (~103 g/L) TS, 0.35 mL of sodium nitrite (100 g/L) TS, 2 mL of ammonium sulfamate (50 g/L) TS, 5 mL of \( N-(1-naphthyl) \) ethylenediamine hydrochloride (1 g/L) TS and 1 mL of ethanol (~750 g/L) TS. Transfer this solution quantitatively to a 50.0 mL volumetric flask, dilute to volume with water R and allow to stand for 30 minutes.
Prepare reference solutions (B)–(F) representing respectively 0.005% (m/v), 0.01% (m/v), 0.02% (m/v) and 0.06% (m/v) of chloroaniline in the test sample as follows: Dilute 1.0 mL, 2.0 mL, 4.0 mL, 10.0 mL and 12.0 mL of a solution containing 10 μg per mL of chloroaniline R in hydrochloric acid (200 g/L) TS to 20 mL with water R. Then add 10.0 mL of water R. Add rapidly and with thorough mixing after each addition: 5 mL of hydrochloric acid (~103 g/L) TS, 0.35 mL of sodium nitrite (100 g/L) TS, 2 mL of ammonium sulfamate (50 g/L) TS, 5 mL of N-(1-naphthyl) ethylenediamine hydrochloride (1 g/L) TS and 1 mL of ethanol (~750 g/L) TS. Transfer each solution quantitatively to a 50.0 mL volumetric flask, dilute to volume with water R and allow to stand for 30 minutes.

Measure the absorbance (1.6) of solutions (A)–(F) in a 1 cm layer at the maximum at about 556 nm. Plot a calibration curve for solutions (B)–(F). Determine the concentration of chloroaniline from the calibration curve.

The content of p-chloroaniline is not more than 0.05% (m/v), calculated with reference to chlorhexidine gluconate solution.

**Related substances**

Prepare fresh solutions and perform the tests without delay.

Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (25 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 µm). Maintain the column at 30°C.

Use the following conditions for gradient solution:

- **Mobile phase A:** Mix 20 volumes of a 0.1% (v/v) solution of trifluoroacetic acid R in acetonitrile R and 80 volumes of a 0.1% (v/v) solution of trifluoroacetic acid R in water R;

- **Mobile phase B:** Mix 10 volumes of a 0.1% (v/v) solution of trifluoroacetic acid R in water R and 90 volumes of a 0.1% (v/v) solution of trifluoroacetic acid R in acetonitrile R.

Use the following 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>0–2</td>
<td>100</td>
<td>0</td>
<td>Isocratic</td>
</tr>
<tr>
<td>2–32</td>
<td>100 to 80</td>
<td>0 to 20</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>32–37</td>
<td>80</td>
<td>20</td>
<td>Isocratic</td>
</tr>
<tr>
<td>37–47</td>
<td>80 to 70</td>
<td>20 to 30</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>47–54</td>
<td>70</td>
<td>30</td>
<td>Isocratic</td>
</tr>
<tr>
<td>54–56</td>
<td>70 to 100</td>
<td>30 to 0</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>56–66</td>
<td>100</td>
<td>0</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

Prepare the following solutions in mobile phase A: For solution (1) transfer 1.0 mL of the preparation to be examined to a 100 mL volumetric flask and dilute to volume. For solution (2) transfer 1.0 mL of solution (1) to a 100 mL volumetric flask and dilute to volume. For
solution (3) dissolve the contents of a vial of chlorhexidine for system suitability RS (containing impurities A, B, F, G, H, I, J, K, L, N and O) in 1.0 mL.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 254 nm.

Inject alternately 10 µL each of solutions (1), (2) and (3).

Use the chromatogram supplied with chlorhexidine for system suitability RS and the chromatogram obtained with solution (3) to identify the peaks due to impurities A, B, F, G, H, I, J, K, L, N and O.

Relative retention With reference to chlorhexidine (retention time = about 35 min):
impurity L = about 0.23; impurity Q = about 0.24; impurity G = about 0.25;
impurity N = about 0.35; impurity B = about 0.36; impurity F = about 0.5;
impurity A = about 0.6; impurity H = about 0.85; impurity O = about 0.90;
impurity I = about 0.91; impurity J = about 0.96; and impurity K = about 1.4.

The test is not valid unless in the chromatogram obtained with solution (3):
• the resolution between the peaks due to impurities L and G is at least 3.0;
• the peak-to-valley ratio (Hp/Hv) is at least 2.0, where Hp = height above the baseline of the peak due to impurity B and Hv = the height above the baseline of the lowest point of the curve separating this peak from that due to impurity N.

In the chromatogram obtained with solution (1):
• the area of any peak corresponding to impurity N is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);
• the area of any peak corresponding to impurity H is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);
• the areas of any peak corresponding to either impurities A, J or K is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);
• the sum of the areas of any peaks corresponding to impurities I and O is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);
• the area of any peak corresponding to impurity G is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);
• the areas of any peak corresponding to either impurities B, F, L or Q is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);
• the areas of any peak corresponding to either impurities A, J or K is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);
• the area of any other impurity peak is not greater than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.10%);
• the sum of the areas of all impurity peaks is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3.0%). Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Assay. Determine the weight per mL (13.1) of the preparation to be examined. Transfer about 1 g, accurately weighed, to a 250 mL beaker and add 50 mL of anhydrous acetic acid R.

Titrate with 0.1 M 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 22.44 mg of C₃₄H₅₄Cl₂N₁₀O₁₄·
Impurities

A. 1-(4-chlorophenyl)-5-[6-[(cyanocarbamimidoyl)amino]hexyl]biguanide,

B. 1-[[6-[[[(4-chlorophenyl)carbamimidoyl]carbamimidoyl]amino]hexyl]carbamimidoyl]urea,

E. 1-(4-chlorophenyl)guanidine,

F. 1-(4-chlorophenyl)urea,

G. 1-(6-aminohexyl)-5-(4-chlorophenyl)biguanide,

H. 1,1’-[iminobis(carbonimidoyliminohexane-6,1-diyl)]bis[5-(4-chlorophenyl)biguanide],
I. unknown structure,

J. 1-(4-chlorophenyl)-5-[[4-[(4-chlorophenyl)amino]-6-[(1S,2R,3R,4R)-1,2,3,4,5-pentahydroxypentyl]-1,3,5-triazin-2-yl]amino]hexy]biguanide,

K. 1-(4-chlorophenyl)-3-[[6-[[((4-chlorophenyl)carbamimidoyl]carbamimidoyl]amino]hexyl]carbamimidoyl]urea,

L. (5R,6S)-2-[(4-chlorophenyl)amino]-5-hydroxy-6-[(1R,2R)-1,2,3-trihydroxypropyl]-5,6-dihydro-4H-1,3-oxazin-4-one,

M. 5-(4-chlorophenyl)-5'-phenyl-1,1'-(hexane-1,6-diyl)dibiguanide,
N. 1-[6-(carbamimidoylamino)hexyl]-5-(4-chlorophenyl)biguanide,

O. 5-(2-chlorophenyl)-5′-(4-chlorophenyl)-1,1′-(hexane-1,6-diyl)dibiguanide,

P. 4-chloroaniline,

Q. unknown structure.

Reference substances to be established
chlorhexidine for system suitability RS

Reagents to be added to Reagents, test solutions and volumetric solutions

Sodium hydroxide (~420 g/L) TS.
A solution of sodium hydroxide R containing about 420 g/L of NaOH.

Titan yellow paper R
Impregnate filter paper with titan yellow TS. Allow to dry at room temperature.

Sulfuric acid (~98 g/L) TS.
Procedure. Add 55 mL of sulfuric acid (~1760 g/L) TS to sufficient water to produce 1000 mL; 
$d \sim 1.063$.

Hydrochloric acid (~103 g/L) TS
Hydrochloric acid (~420 g/L) TS, dilute with water to contain 103 g of HCl in 1000 mL.

Hydrochloric acid (~200 g/L) TS
Hydrochloric acid (~420 g/L) TS, dilute with water to contain 200 g of HCl in 1000 mL.
Chlorhexidine digluconate topical solution
(Chlorhexidini digluconatis solutio topicales)

This is a draft proposal for The International Pharmacopoeia (Working document QAS/16.669, June 2016). The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/. 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. Antiseptic.

Storage. Chlorhexidine digluconate topical solution should be kept in a well-closed container, protected from light.

Additional information. Strength in the current WHO Model List of Essential Medicines (EML): 5% (digluconate) and 7.1% (digluconate), delivering 4% chlorhexidine (for umbilical cord care); strengths in the current EML for children: 5% (digluconate) and 7.1% (digluconate), delivering 4% chlorhexidine (for umbilical cord care).

Definition. Chlorhexidine digluconate topical solution is a solution of Chlorhexidine digluconate solution in a suitable vehicle. It contains not less than 90.0% and not more than 110.0% of the amount of chlorhexidine digluconate \( (C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7) \) stated on the label.

Identity tests
A. 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 50 volumes of dehydrated ethanol R, 10 volumes of ethyl acetate R, 10 volumes of ammonia (~260 g/L) TS R and 30 volumes of water R as the mobile phase. Apply separately to the plate 2 μL of each of the following 2 solutions in water R. For solution (A) dilute a quantity of the topical solution to obtain a solution containing 20 mg of chlorhexidine digluconate per mL. For solution (B) use a solution containing 10 mg of potassium gluconate R per mL. After removing the plate from the chromatographic chamber heat the plate for 20 minutes at 110°C and allow the plate to cool. Spray with ammonium molybdate/ceric sulfate/sulfuric acid TS. Heat the plate for 10 minutes at 110°C. Examine the chromatogram in daylight.

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

B. Transfer a quantity of the topical solution containing 5 mg of chlorhexidine digluconate into a 500 mL volumetric flask and dilute to volume with water R. The absorption spectrum (1.6) of the resulting solution, when observed between 200 nm and 320 nm, exhibits two maxima at about 231 nm and 255 nm, and two minima at about 218 nm and 242 nm.

C. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given 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 chlorhexidine in the chromatogram obtained with solution (2).

pH value (1.13). 5.0–7.0.
Impurity P (4-Chloroaniline)

Prepare fresh solutions and perform the tests without delay. Carry out the test as described under 1.14.4 *High-performance liquid chromatography* using the same chromatographic conditions as described under “Assay”.

Prepare the following solutions in mobile phase A:

For solution (1) transfer an amount of the topical solution, equivalent to 40.0 mg chlorhexidine digluconate to a 100 mL volumetric flask, and dilute to volume.

For solution (2) use 1.0 μg of 4-chloroaniline R per mL.

For solution (3) prepare a solution that contains 50 μg per mL of chlorhexidine diacetate RS and 1 μg per mL 4-chloroaniline R.

Inject 50 μL of solution (3). In the chromatogram obtained with solution (3) the peak due to 4-chloroaniline is eluted at a relative retention of about 1.3 with reference to chlorhexidine (retention time about 6 minutes). The test is not valid unless the resolution between the peaks due to 4-chloroaniline and chlorhexidine is at least 3.0.

Inject alternately 50 μL of each of solutions (1) and (2).

Measure the areas of the peaks corresponding to 4-chloroaniline obtained in the chromatograms of solutions (1) and (2).

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to 4-chloroaniline is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.05% (m/v) with reference to the concentration of chlorhexidine digluconate in the topical solution).

**Assay**

Prepare fresh solutions and perform the tests without delay.

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 particles of silica gel, the surface of which has been modified with chemically-bonded octadecysilane groups (5 μm).\(^\text{1}\)

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>0–9</td>
<td>100</td>
<td>0</td>
<td>Isocratic</td>
</tr>
<tr>
<td>9–10</td>
<td>100 to 45</td>
<td>0 to 55</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>10–15</td>
<td>45</td>
<td>55</td>
<td>Isocratic</td>
</tr>
<tr>
<td>15–16</td>
<td>45 to 100</td>
<td>55 to 0</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>16–21</td>
<td>100</td>
<td>0</td>
<td>Isocratic</td>
</tr>
</tbody>
</table>

\(^\text{1}\) Symmetry C18, 250 mm x 4.6 mm – 5 μm was found suitable.
Operate with a flow rate of 1.5 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 239 nm.

Maintain the column temperature at 40°C.

Prepare the following solutions:

For solution (1) transfer an amount of the topical solution, equivalent to about 40 mg chlorhexidine digluconate, accurately weighed, to a 100 mL volumetric flask, and dilute to volume with methanol R. Further dilute a 10 mL portion of this solution with mobile phase A to 50 mL.

For solution (2) use 50 μg of chlorhexidine diacetate RS per mL in mobile phase A.

For solution (3) prepare a solution that contains 50 μg per mL of chlorhexidine diacetate RS and 1 μg per mL 4-chloroaniline R in mobile phase A.

Inject 50 μL of solution (3).

In the chromatogram obtained with solution (3) the peak due to 4-chloroaniline is eluted at a relative retention of about 1.3 with reference to chlorhexidine (retention time about 6 minutes). The assay is not valid unless the resolution between the peaks due to 4-chloroaniline and chlorhexidine is at least 3.0.

Inject alternately 50 μL of each of solutions (1) and (2).

Measure the areas of the peaks corresponding to chlorhexidine obtained in the chromatograms of solutions (1) and (2). Determine the weight per mL (1.3.1) and calculate the percentage content of chlorhexidine digluconate, weight in volume, in the topical solution using the declared content of chlorhexidine in chlorhexidine diacetate RS (C22H30Cl2N10·2C2H4O2). Each mg of chlorhexidine (C22H30Cl2N10) is equivalent to 1.776 mg of chlorhexidine digluconate (C22H30Cl2N10·2C6H4ClO7).

Impurities. The impurity limited by the requirements of this monograph is impurity P listed in the monograph for Chlorhexidine digluconate solution.

Reagents to be added to Reagents, test solutions and volumetric solutions

Potassium gluconate R
C6H11KO7

Reference substances to be established

Chlorhexidine diacetate RS
4-chloroaniline RS
Medroxyprogesterone acetate  
*(Medroxyprogesteroni acetas)*

This is a draft proposal of a revised monograph for *The International Pharmacopoeia* (Working document QAS/16.647, June 2016).

The working document with line numbers and tracked changes is available for comment at [www.who.int/medicines/areas/quality_safety/quality_assurance/projects](http://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.

[Note from the Secretariat. Following information received from our custodian centre for International Chemical Reference Substances (ICRS), the European Directorate for the Quality of Medicines & Healthcare, it is proposed to revise the monograph on Medroxyprogesterone acetate.]

[Note from the editor. In accordance with WHO editorial policy the text reproduced below does not include tracked changes. Changes from the current monograph are indicated by insert and delete in the working document available at the above-mentioned web address.]

\[
\begin{align*}
\text{C}_{24}\text{H}_{34}\text{O}_4 & \quad \text{Relative molecular mass.} \quad 386.5 \\
\text{Chemical name.} & \quad 6α\text{-methyl-3,20-dioxopregn-4-en-17-yl acetate; (6α)-17-(acetyloxy)-6-}
\text{methylpregn-4-ene-3,20-dione; 17-hydroxy-6α-methylprogesterone acetate; CAS Reg. No.} \\
& \quad 71-58-9. \\
\text{Description.} & \quad \text{A white or almost white, crystalline powder.} \\
\text{Solubility.} & \quad \text{Practically insoluble in water; soluble in acetone R and dioxan R; slightly soluble in} \\
& \quad \text{ethanol (~750 g/L) TS, methanol R and ether R.} \\
\text{Category.} & \quad \text{Progestogen.} \\
\text{Storage.} & \quad \text{Medroxyprogesterone acetate should be kept in a tight container, protected from} \\
& \quad \text{light.} \\
\text{Requirements} & \quad \text{Definition.} \quad \text{Medroxyprogesterone acetate contains not less than 97.0% and not more than the} \\
& \quad \text{equivalent of 103.0% of C}_{24}\text{H}_{34}\text{O}_4, \text{calculated with reference to the dried substance.} \\
\text{Identity tests} & \quad \text{Either test A or tests B and C may be applied.}
\end{align*}
\]
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 medroxyprogesterone acetate RS or with the reference spectrum of medroxyprogesterone acetate.

B. Carry out test B.1 or, where UV detection is not available, test B.2.

B.1  Carry out the test as described under 1.14.1, Thin-layer chromatography using silica R5 as the coating substance and a mixture of 10 volumes of dichloromethane R and 1 volume of ethyl acetate R as the mobile phase. Apply separately to the plate 10 µL of each of the following three solutions in dichloromethane R. For solution (A) use 2.5 mg of Medroxyprogesterone acetate per mL. For solution (B) use 2.5 mg of medroxyprogesterone acetate RS per mL. After removing the plate from the chromatographic chamber heat it at 120°C for 30 minutes, spray with 4-toluenesulfonic acid/ethanol TS and heat further at 120°C for 10 minutes. Allow the plate to cool and examine the chromatogram in ultraviolet light (365 nm).

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

B.2 Carry out the test as described under 1.14.1 Thin-layer chromatography using the conditions described under test B.1, but spray the plate with a mixture of equal volumes of sulfuric acid R and ethanol (~750 g/L) TS and heat further at 120°C for 10 minutes. Allow the plate to cool and examine the chromatogram in daylight.

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

C. Use 20 mg; it yields the reaction described under 2.1 General identification tests as characteristic of acetylated substances.

Specific optical rotation. Use a 10 mg/mL solution in acetonitrile R; [a]_{20°C} = +47° to +53°.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at 105°C for 3 hours; it loses not more than 10 mg/g.

Impurity F

A. 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 particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (3 µm).

As the mobile phase use a solution prepared as follows: mix 44 volumes of water R and 56 volumes of acetonitrile R.

For solution (1) dissolve 20 mg of Medroxyprogesterone acetate in 5.0 mL of acetonitrile R and dilute to 10.0 mL with water R. For solution (2) dilute 0.5 volume of solution (1) to 100 volumes with the mobile phase. For solution (3) use 0.2 mg of medroxyprogesterone acetate RS and 0.01 mg of medroxyprogesterone acetate impurity F RS per mL mobile phase.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 200 nm.

Inject 25 µL of solution (1), (2) and (3). In the chromatogram obtained with solution (3) impurity F is eluted at a relative retention of about 1.8 with reference to medroxyprogesterone acetate (retention time about 8 minutes).
In the chromatogram obtained with solution (1) the area of any peak corresponding to impurity F, when multiplied by a correction factor of 1.8, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

**Related substances**

Prepare fresh solutions and perform the tests without delay.

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

Maintain the column temperature at 60°C.

As the mobile phase, use a solution prepared as follows: mix 12 volumes of tetrahydrofuran R, 23 volumes of acetonitrile R and 65 volumes of water R and filter.

Prepare the following solutions in a dissolution solvent prepared by mixing equal volumes of acetonitrile R and water R.

For solution (1) dissolve 20 mg of Medroxyprogesterone acetate and dilute to 10.0 mL.

For solution (2) dilute 1.0 mL of solution (1) to 100.0 mL.

For solution (3) dilute 1.0 mL of solution (2) to 10.0 mL.

For solution (4) dissolve 4 mg of medroxyprogesterone acetate for system suitability RS (containing medroxyprogesterone acetate and the impurities A, B, C, D, E, G and I) and dilute to 2.0 mL.

Operate with a flow rate of 0.9 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 254 nm.

Inject separately 20 µL of solution (1), (2), (3) and (4). Record the chromatogram for about twice the retention time of medroxyprogesterone acetate in solution (2).

Use the chromatogram supplied with medroxyprogesterone acetate for system suitability RS and the chromatogram obtained with solution (4) to identify the peaks due to impurities A, B, C, D, E, G and I. The impurities are eluted at the following relative retention with reference to the peak of medroxyprogesterone acetate (retention time about 27 minutes): impurity A about 0.3; impurity I about 0.5; impurity H about 0.65; impurity B about 0.7; impurity C about 0.8; impurity G about 0.85; impurity D about 0.9; impurity E about 0.95.

The test is not valid unless in the chromatogram obtained with solution (4) the resolution factor between the peaks due to impurity G and due to medroxyprogesterone acetate is at least 3.3.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity D is not greater than the area of the principal peak obtained with solution (2) (1.0%);
- the area of any peak corresponding to impurity B is not greater than 0.7 times the area of the principal peak obtained with solution (2) (0.7%);
- the area of any peak corresponding to impurity A, when multiplied by a correction factor of 1.5, is not greater than 3 times of the area of the principal peak obtained with solution (3) (0.3%);
- the area of any peak corresponding to impurity G, when multiplied by a correction factor of 2.6, is not greater than 2 times of the area of the principal peak obtained with solution (3) (0.2%);
- the area of any peak corresponding to impurity C, E or I is not greater than 2 times the area of the principal peak obtained with solution (3) (0.2%).
• the area of any other impurity peak is not greater than the area of the principal peak obtained with solution (3) (0.1%);

• the sum of the areas (corrected where necessary) of all the peaks, other than the principal peak, is not greater than 1.5 times the area of the principal peak obtained with solution (2) (1.5%). Disregard any peak with an area less than 0.5 times the area of the principal peak obtained with solution (3) (0.05%).

Assay
Dissolve about 0.1 g, accurately weighed, in ethanol (~750g/L) TS to produce 100 mL; dilute 1.0 mL of this solution to 100 mL with the same solvent.

Measure the absorbance of the diluted solution in a 1 cm layer at the maximum at about 241 nm and calculate the content of $\text{C}_{24}\text{H}_{34}\text{O}_4$ using the absorptivity value of 42.6 ($\varepsilon_{1\text{cm}} = 426$)

Impurities

A. 6β-hydroxy-6α-methyl-3,20-dioxopregn-4-en-17-yl acetate (6-hydroxy(medroxyprogesterone acetate)),

B. 17-hydroxy-6α-methylpregn-4-ene-3,20-dione (medroxyprogesterone),

C. 6α,17αβ-dimethyl-3,17-dioxo-D-homopregn-4-en-17αβ-yl acetate,

D. 6β-methyl-3,20-dioxopregn-4-en-17-yl acetate (6-epi-medroxyprogesterone acetate),
E. 6-methylidene-3,20-dioxopregn-4-en-17-yl acetate (6-methylidenehydroxyprogesterone acetate),

F. 6α-methyl-3,20-dixo-5β-pregnan-17-yl acetate (4,5β-dihydromedroxyprogesterone acetate),

G. 6-methyl-3,20-dioxopregna-4,6-dien-17-yl acetate (megestrol acetate),

H. 3,20-dioxopregn-4-en-17-yl acetate (hydroxyprogesterone acetate),

I. 17αβ-hydroxy-6α,17αα-dimethyl-D-homopregn-4-ene-3,17-dione.

***
Medroxyprogesterone injection  
(*Medroxyprogesteroni injectio*)

This is a draft proposal of a revised monograph for *The International Pharmacopoeia* (Working document QAS/16.670, June 2016).

The working document with line numbers and tracked changes 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.

(Note from the Secretariat. Following information received from our custodian centre for International Chemical Reference Substances (ICRS), the European Directorate for the Quality of Medicines & HealthCare, it is proposed to revise the monograph on Medroxyprogesterone injection.)

(Note from the editor. In accordance with WHO editorial policy the text reproduced below does not include tracked changes. Changes from the current monograph are indicated by *insert* and *delete* in the working document available at the above-mentioned web address.)

**Category.** Contraceptive.

**Storage.** Medroxyprogesterone injection should be protected from light. On standing solid matter may separate; it should be resuspended before use.

**Additional information.** Strength in the current WHO Model List of Essential Medicines: 150 mg/mL in 1 mL vial.

**Requirements**

Complies with the monograph for *Parenteral preparations*.

**Definition.** Medroxyprogesterone injection is a sterile aqueous suspension of Medroxyprogesterone acetate. It contains not less than 90.0% and not more than 110.0% of the amount of Medroxyprogesterone acetate (C_{24}H_{34}O_{4}) stated on the label.

**Identity tests**

• Either test A alone or tests B and C or tests C and D may be applied.

A. Centrifuge a volume of the injection to be examined containing 50 mg of Medroxyprogesterone acetate. Decant the supernatant liquid and discard. Dry the residue at 105°C for 3 hours and 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 medroxyprogesterone acetate RS or with the reference spectrum of medroxyprogesterone acetate.

B. Carry out test B.1 or, where UV detection is not available, test B.2.

B.1 Carry out the test as described under 1.14.1 Thin-layer chromatography using silica R5 as the coating substance and a mixture of 10 volumes of dichloromethane R and 1 volume of ethyl acetate R as the mobile phase. Apply separately to the plate 10 μL of each of the following three solutions in dichloromethane R. For solution (A) measure a volume of injection to be examined containing about 40 mg of Medroxyprogesterone acetate, add 15 mL of dichloromethane R, shake vigorously for
20 minutes, allow to stand for 30 minutes, add 2.5 g sodium sulphate anhydrous R, shake for 5 minutes and allow to stand for another 10 minutes. For solution (B) use 2.5 mg of medroxyprogesterone acetate RS per mL. After removing the plate from the chromatographic chamber heat it at 120°C for 30 minutes, spray with 4-toluenesulfonic acid/ethanol TS and heat further at 120°C for 10 minutes. Allow the plate to cool and examine the chromatogram in ultraviolet light (365 nm).

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

B.2 Carry out the test as described under 1.14.1 Thin-layer chromatography using the conditions described under test B.1, but spray the plate with a mixture of equal volumes of sulfuric acid R and ethanol (~750 g/L) TS and heat further at 120°C for 10 minutes. Allow the plate to cool and examine the chromatogram in daylight.

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

C. Centrifuge a volume of injection to be examined containing 30 mg of Medroxyprogesterone acetate. Decant the supernatant liquid, dissolve the residue in 5 mL of sulfuric acid R and introduce 5 mL of ethanol (~750 g/L) TS to form an upper layer; a bluish violet ring is formed at the interface of the two layers.

D. See the test described below under “Assay”. The retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that in the chromatogram obtained with solution (2).

**pH.** pH of the injection, 3.0–7.0.

**Impurity F (4,5-Dihydromedroxyprogesterone acetate).** 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 10 volumes of tetrahydrofuran R, 45 volumes of tert-butyl methyl ether R and 45 volumes of hexane R as the mobile phase.

Apply separately to the plate 10 µL of each of the following three solutions in dichloromethane R. For solution (A) accurately measure a volume of injection to be examined containing 300 mg of Medroxyprogesterone acetate, add 15 mL of dichloromethane R, shake vigorously for 20 minutes, allow to stand for 30 minutes, add 10 g sodium sulphate anhydrous R, shake for 5 minutes and allow to stand for another 10 minutes. For solution (B) dilute 0.5 volume of solution (1) to 100 volumes. For solution (C) use 20 mg of medroxyprogesterone acetate RS and 0.1 mg of medroxyprogesterone acetate impurity F RS per mL.

Develop the plate for a distance of about 10 cm. Allow it to dry in air and carry out a second development in the same direction using a freshly prepared mobile phase. After removing the plate from the chromatographic chamber heat it at 100°C to 105°C for 30 minutes and spray with 4-toluenesulfonic acid/ethanol TS. Heat again at 120°C for 10 minutes, allow to cool and examine the chromatogram in ultraviolet light (365 nm).

In the chromatogram obtained with solution (C) impurity F has a Rf value of about 0.66 and medroxyprogesterone acetate an Rf value of about 0.56. The test is not valid unless the chromatogram obtained with solution (C) shows two clearly separated spots. In the chromatogram obtained with solution (A) any spot due to impurity F is not more intense than the corresponding spot in the chromatogram obtained with solution (B) (0.5%).

**Related substances**

Prepare fresh solutions and perform the tests without delay.
Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under “Assay”.

Prepare the following solutions with the mobile phase. For solution (1) dilute a suitable volume of the injection to be examined to obtain a concentration of 0.4 mg of Medroxyprogesterone acetate per mL. For solution (2) dilute 1.0 mL of solution (1) to 100.0 mL. For solution (3) dissolve 4 mg of medroxyprogesterone acetate for system suitability RS (containing medroxyprogesterone acetate and the impurities A, B, C, D, E, G and I) and dilute to 2.0 mL. For solution (4) use 3.65 μg of methyl hydroxybenzoate R and 0.4 μg of propyl hydroxybenzoate R per mL.

Inject separately 20 μL of solution (1), (2), (3) and (4). Record the chromatogram for about twice the retention time of medroxyprogesterone acetate in solution (2).

Use the chromatogram supplied with medroxyprogesterone acetate for system suitability RS and the chromatogram obtained with solution (4) to identify the peaks due to impurities A, B, C, D, E, G and I. The impurities are eluted at the following relative retention with reference to the peak of medroxyprogesterone acetate (retention time about 27 minutes): impurity A about 0.3; impurity I about 0.5; impurity H about 0.65; impurity B about 0.7; impurity C about 0.8; impurity G about 0.85; impurity D about 0.9; impurity E about 0.95.

The test is not valid unless the resolution factor between the peaks due to medroxyprogesterone acetate and due to impurity G is at least 3.3.

In the chromatogram obtained with solution (1):
• the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);
• the sum of the areas of all peaks, other than the principal peak, is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak due to hydroxybenzoate derivatives in the chromatogram obtained with solution (4). Disregard any peak with an area less than 0.05 times the area of the principal peak 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 × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 μm).

As the mobile phase use a solution prepared as follows: mix 100 volumes of tetrahydrofuran R, 350 volumes of acetonitrile R and 500 volumes of water R.

Prepare the following solutions in the mobile phase. For solution (1) dilute a suitable volume of the injection to be examined to obtain a concentration of 40 μg of Medroxyprogesterone acetate per mL. For solution (2) dissolve 10 mg of medroxyprogesterone acetate RS in 50 mL. Dilute 5 mL of this solution to 25 mL.

Operate with a flow rate of 0.9 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 241 nm.

Inject separately 20 μL of solution (1) and (2).

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2) and calculate the percentage content of medroxyprogesterone acetate (C_{24}H_{34}O_{4}), using the declared content of C_{24}H_{34}O_{4} in medroxyprogesterone acetate RS.
Clindamycin palmitate hydrochloride  
(*Clindamycini palmitas hydrochloridum*)

This is a draft proposal of a monograph for *The International Pharmacopoeia* 

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: schmidt@who.int.

**Molecular formula.** $C_{34}H_{63}ClN_2O_6S\cdot HCl$

**Relative molecular mass.** 699.85

**Graphic formula**

![Graphic formula](image)

**Chemical name.** L-threo-α-D-galacto-Octopyranoside, methyl 7-chloro-6,7,8- trideoxy-6-[(1-methyl-4-propyl-2-pyrrolidinyl)-carbonyl]amino]-1-thio-2-hexadecanoate, monohydrochloride, (2S-trans)-; Methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-trans-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-L-threo-α-D-galacto-octopyranoside 2-palmitate monohydrochloride; CAS Reg. No. 25507-04-4.

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

**Solubility.** Freely soluble in ethanol and in dichloromethane; soluble in water.

**Category.** Antibacterial.

**Storage.** Clindamycin palmitate hydrochloride should be preserved in a tightly closed container.

**Additional information.** Clindamycin palmitate hydrochloride is a semi-synthetic product derived from a fermentation product.

**Requirements**

**Definition.** Clindamycin palmitate hydrochloride contains not less than 91.0% and not more than 102.0% of $C_{34}H_{63}ClN_2O_6S\cdot HCl$, calculated with reference to the anhydrous substance.

**Identity tests**

* Either tests A and D or tests B, C and D 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 clindamycin palmitate hydrochloride RS or with the reference spectrum of clindamycin palmitate hydrochloride.
B. Carry out the test as described under **1.14.4 High-performance liquid chromatography** using the conditions given under “Assay”. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

C. Dissolve about 10 mg in 2 mL of hydrochloric acid (~200 g/L) TS and heat on a water-bath for 3 minutes. Add 3 mL of sodium carbonate (106 g/L) TS and 1 mL of sodium nitroprusside (20 g/L) TS. A violet-red colour develops [test to be verified].

D. A 10 mg/mL solution yields reaction B described under **2.1 General identification tests** as characteristic of chlorides.

**Water.** Determine as described under **2.8 Determination of water by the Karl Fischer method**, Method A, using 0.1 g of the substance; the water content is not more than 30 mg/g.

**pH value.** pH of a 10 mg/mL solution in carbon-dioxide-free water R, 2.8–3.8.

**Sulfated ash.** Not more than 5 mg/g.

**Related substances**

Carry out the test as described under **1.14.4 High-performance liquid chromatography** using a column (25 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octylsilyl groups (5 μm).\(^1\)

Use the following conditions for gradient elution:
- mobile phase A: Ammonium acetate (~0.40 g/L) TS - acetonitrile R (50:50);
- mobile phase B: Acetonitrile R.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–30</td>
<td>100 to 0</td>
<td>0 to 100</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>30–80</td>
<td>0</td>
<td>100</td>
<td>Isocratic</td>
</tr>
<tr>
<td>80–81</td>
<td>0 to 100</td>
<td>100 to 0</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>81–90</td>
<td>100</td>
<td>0</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

Prepare the following solutions in methanol R. For solution (1) dissolve 100 mg of clindamycin palmitate hydrochloride and dilute to 10.0 mL. For solution (2) dilute 2.0 mL of solution (1) to 100 mL. For solution (3) dissolve about 74 mg clindamycin palmitate hydrochloride RS (containing clindamycin palmitate hydrochloride and impurity A) and dilute to 10.0 mL.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 230 nm.

Inject alternately 20 μL each of solution (1), (2) and (3).

In the chromatogram obtained with solution (3) the retention time of clindamycin palmitate is about 37 minutes. The test is not valid unless the resolution between the peaks due to clindamycin palmitate and impurity A (relative retention about 0.9) is at least 3.0.

In the chromatogram obtained with solution (1):

- the area of any impurity peak is not more than the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);
- the sum of the areas of all impurity peaks is not more than 3.5 times the area of the principal peak in the chromatogram obtained with solution (2) (7.0%). Disregard any peak with an area less than 0.025 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

\(^1\) Agilent Zorbax Elipse XDB- C8 has been found suitable.
Assay

[The method is currently under validation. Some parameters need to be amended.]

Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (25 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octylsilyl groups (5 μm).²

As the mobile phase use a mixture of 10 volumes of ammonium acetate (~0.40 g/L) TS and 90 volumes of acetonitrile R.

Prepare the following solutions in mobile phase. For solution (1) transfer 50.0 mg of clindamycin palmitate hydrochloride into a 50 mL volumetric flask and dilute to volume. For solution (2) dissolve 50 mg of clindamycin palmitate hydrochloride RS (containing clindamycin palmitate hydrochloride and impurity A) into a 50 mL volumetric flask and dilute to 50.0 mL.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 230 nm.

Inject alternately 20 μL each of solutions (1) and (2). The retention time of clindamycin palmitate is about x minutes. The assay is not valid unless in the chromatogram obtained with solution (2) the resolution between the peaks due to clindamycin palmitate and to impurity A (relative retention time is about x) is at least x.

Measure the areas of the peaks corresponding to clindamycin palmitate obtained in the chromatograms from solutions (1) and (2) and calculate the percentage content of clindamycin palmitate hydrochloride (C₃₄H₆₃ClN₂O₆S.HCl), using the declared content of clindamycin palmitate hydrochloride (C₃₄H₆₃ClN₂O₆S.HCl) in clindamycin palmitate hydrochloride RS.

Impurities

A. L-threo-α-D-galacto-Octopyranoside, methyl 7-chloro-6,7,8-trideoxy-6-[[{1- methyl-4-ethyl-2-pyrrolidinyl}-carbonyl]amino]-1-thio-2-hexadecanoate, (2S-trans)-; Methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-trans-4-ethyl-L-2- pyrrolidine carboxamido)-1- thio-L-threo-α-D-galacto-octopyranoside 2-palmitate (clindamycin B palmitate) (synthesis-related impurity)

Reagents to be established

Ammonium acetate (~0.40 g/L) TS
A solution of ammonium acetate R containing about 0.385 g of C₂H₂N₂O₂ per litre (approximately 0.005 mol/L).

Docusate sodium R
C₂₀H₃₇NaO₇S
A commercially available reagent of suitable grade.

² Agilent Zorbax Elipse XDB- C8 has been found suitable.
Clindamycin palmitate for oral suspension
*(Clindamycini palmitas ad suspensionem peroralem)*

This is a draft proposal of a monograph for *The International Pharmacopoeia* (Working document QAS/16.655, July 2016).

The working document with line numbers is available for comment at [www.who.int/medicines/areas/quality_safety/quality_assurance/projects](http://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: schmidt@who.int.

**Category.** Antibacterial.

**Storage.** Clindamycin palmitate hydrochloride for oral suspension should be kept in a tightly closed container.

**Additional information.** Strength in the current WHO Model List of Essential Medicines (EML): 75 mg/5 mL (as palmitate). Strengths in the current WHO EML for Children: 75 mg/5 mL (as palmitate).

**Labelling.** The designation on the container of clindamycin palmitate for oral suspension should state that the active ingredient is clindamycin palmitate hydrochloride and the quantity should be indicated in terms of equivalent amount of clindamycin.

**Requirements**

Complies with the monograph for *Liquid preparations for oral use*; the powder complies with the section of the monograph entitled “*Powders for oral solutions, oral suspensions and oral drops*”.

**Definition.** Clindamycin palmitate for oral suspension is a suspension of Clindamycin palmitate hydrochloride in a suitable vehicle, which may be flavoured. It is prepared from the powder as stated on the label just before issue for use. When freshly constituted the oral suspension contains not less than 90.0% and not more than 110.0% of the labelled amount of clindamycin (C_{18}H_{33}ClN_{2}O_{5}S).

**Identity tests**

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

**pH value.** pH of a solution constituted as directed in the labelling, 2.5–5.0.

**Loss on drying.** Dry the powder for oral suspension to constant mass at 60°C under reduced pressure; it loses not more than 20 mg/g.

**Related substances**

Use the oral suspension immediately after preparation.
Carry out the test as described under 1.14.4 High-performance liquid chromatography using a column (25 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octylsilyl groups (5 μm).¹

Use the following conditions for gradient elution:

- mobile phase A: Ammonium acetate (≈0.40 g/L) TS – acetonitrile R (50:50);
- mobile phase B: Acetonitrile R.

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<tr>
<td>81–90</td>
<td>100</td>
<td>0</td>
<td>Re-equilibration</td>
</tr>
</tbody>
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Prepare the following solutions in methanol R. For solution (1) transfer a quantity of the oral suspension, equivalent of about 57 mg of clindamycin to a 10 mL volumetric flask and dilute to volume. For solution (2) dilute 2.0 ml of solution (1) to 100.0 mL. For solution (3) dissolve about 74 mg clindamycin palmitate hydrochloride RS (containing clindamycin palmitate hydrochloride and impurity A) and dilute to 10.0 mL.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 230 nm.

Inject alternately 20 μL each of solution (1), (2) and (3).

In the chromatogram obtained with solution (3) the retention time of clindamycin palmitate is about 37 minutes. The test is not valid unless the resolution between the peaks due to clindamycin palmitate and impurity A (relative retention time about 0.9 ) is at least 3.0.

In the chromatogram obtained with solution (1):

- the area of any impurity peak is not more than the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

**Assay**

*The method is currently under validation. Some parameters need to be amended.*

Use the oral suspension immediately after preparation.

Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (25 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octylsilyl groups (5 μm).²

As the mobile phase use a mixture of 10 volumes of ammonium acetate (≈0.40 g/L) TS and 90 volumes of acetonitrile R.

Prepare the following solutions in the mobile phase. For solution (1) dissolve a quantity of the oral suspension, equivalent to about 225 mg of clindamycin, accurately weighed, and dilute to 50.0 mL, filter and use the filtrate. For solution (2) dissolve about 74 mg clindamycin palmitate hydrochloride RS (containing clindamycin palmitate hydrochloride and impurity A) and dilute to 10.0 mL.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 230 nm.

1 Agilent Zorbax Elipse XDB- C8 (4.6 × 250 mm, 5 μm) has been found suitable.
2 Agilent Zorbax Elipse XDB- C8 has been found suitable.
Inject alternately 20 μL each of solutions (1) and (2). The retention time of clindamycin palmitate is about x minutes. The assay is not valid unless in the chromatogram obtained with solution (2) the resolution between the peaks due to clindamycin palmitate and impurity A (relative retention time is about x) is at least x.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2). Determine the weight per mL (1.3.1) of the oral suspension and calculate the percentage content of clindamycin (C_{18}H_{33}ClN_{2}O_{5}S) in the oral suspension, using the declared content of clindamycin palmitate hydrochloride (C_{34}H_{63}ClN_{2}O_{6}S.HCl) in clindamycin palmitate hydrochloride RS. Each mg of clindamycin palmitate hydrochloride (C_{34}H_{63}ClN_{2}O_{6}S.HCl) is equivalent to 0.607 mg clindamycin (C_{18}H_{33}ClN_{2}O_{5}S).

**Impurities**

The impurities limited by the requirements of this monograph include impurity A listed in the monograph for Clindamycin palmitate hydrochloride.
Clindamycin phosphate
(Clindamycini phosphas)

This is a draft proposal of a revised monograph for The International Pharmacopoeia (Working document QAS/16.678, August 2016).

The working document with line numbers and tracked changes 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.

[Note. In accordance with WHO editorial policy the text reproduced below does not include tracked changes. Changes from the current monograph are indicated by insert and delete in the working document available at the above-mentioned web address.]

\[C_{18}H_{34}ClN_{2}O_{8}PS\]

Relative molecular mass. 505.0

Chemical name

Description. A white or almost white, crystalline powder.

Solubility. Freely soluble in water; very slightly soluble in ethanol (~750 g/L) TS and acetone R, practically insoluble in dichloromethane R.

Category. Antibacterial.

Storage. Clindamycin phosphate should be kept in a tightly closed container.

Additional information. Clindamycin phosphate is slightly hygroscopic and may exhibit polymorphism. It is a semi-synthetic product derived from a fermentation product.

Requirements
Clindamycin phosphate contains not less than 96.0% and not more than 102.0% of \(C_{18}H_{34}ClN_{2}O_{8}PS\), calculated with reference to the anhydrous substance.

• Either tests A and D or tests B, C and D 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 clindamycin phosphate RS or with the reference spectrum of clindamycin phosphate. If the spectra thus obtained are not concordant repeat the test using the residues obtained. Separately dissolve the test substance and clindamycin phosphate RS in a small amount of water R and heat until the substances are completely dissolved. Evaporate to dryness under reduced pressure and dry the residues at 100–105°C for 2 hours. The infrared absorption spectrum is concordant with the spectrum obtained from clindamycin phosphate RS.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography using silica gel R3 as the coating substance and a mixture of 6 volumes of 1-butanol R, 2 volumes of water and 2 volumes of glacial acetic acid R as the mobile phase. Apply separately to the plate 5 μL of each of 3 solutions in methanol R containing (A) 2.0 mg of Clindamycin phosphate per mL, (B) 2.0 mg of clindamycin phosphate RS and for solution (C) dissolve 10 mg of lincomycin hydrochloride RS in 5 mL of solution B. After removing the plate from the chromatographic chamber allow it to dry at 105°C for 30 minutes and spray with potassium permanganate (1 g/L) TS. Examine the chromatogram in daylight. The principal spot obtained with solution A corresponds in position, appearance and intensity to that obtained with solution B. The test is not valid unless the chromatogram obtained with solution C shows two clearly separated spots.

C. Dissolve 10 mg in 2 mL of hydrochloric acid (~70 g/L) TS and heat directly in a flame for 1 minute; a disagreeable sulfurous odour is perceptible. Cool, add 4 mL of sodium carbonate (75 g/L) TS and 0.5 mL of sodium nitroprusside (45 g/L) TS; a violet-red ring is formed that fades quickly.

D. Boil 0.1 g under a reflux condenser with a mixture of 5 mL of sodium hydroxide (~400 g/L) TS and 5 mL of water for 90 minutes. Cool and add 5 mL of nitric acid (~1000 g/L) TS. Extract with three 15 mL quantities of dichloromethane R and discard the extracts. Filter the aqueous layer through a paper filter; the filtrate yields reaction B described under 2.1 General identification tests as characteristic of orthophosphates.

Specific optical rotation. Use a 10 mg/mL solution and calculate with reference to the anhydrous substance; [α]D20°C^20°C = +115° to +130°.

Clarity and colour of solution. Dissolve 1.00 g in carbon dioxide-free water R. Heat gently if necessary. Cool and dilute to 25.0 mL with carbon dioxide-free water R. This solution is clear and colourless, when analysed as described under 1.11.2 Degree of coloration of liquids, Method II.

[Note from the Secretariat. The chapter 1.11 Colour of liquids is currently under revision. Reference is already made to the new test procedure to be added under the section 1.11.2 Degree of coloration of liquids.]

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, method A, using 0.2 g of the substance; the water content is not more than 0.050 g/g.

pH value. pH of a 10 mg/mL solution in carbon-dioxide-free water R, 3.5–4.5.

Related substances. Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (15 cm × 4.6 mm) packed with end-capped particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 μm).^1

^1 A Symmetry C18 column was found suitable.
Use the following conditions for gradient elution:

- mobile phase A: 21 volumes of acetonitrile for chromatography R and 79 volumes of phosphate buffer pH 6.0;
- mobile phase B: 60 volumes of acetonitrile for chromatography R and 40 volumes of phosphate buffer pH 6.0.

Prepare the phosphate buffer pH 6.0 by dissolving 13.6 g of potassium dihydrogen phosphate R in 750 mL of water R, adjust the pH to 6.0 with potassium hydroxide (~450 g/L) TS and dilute to 1000 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>
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<tr>
<td>0–13</td>
<td>100</td>
<td>0</td>
<td>Isocratic</td>
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<tr>
<td>13–18</td>
<td>100 to 50</td>
<td>0 to 50</td>
<td>Linear gradient</td>
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<tr>
<td>18–39</td>
<td>50</td>
<td>50</td>
<td>Isocratic</td>
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<tr>
<td>39–40</td>
<td>50 to 100</td>
<td>50 to 0</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>40–55</td>
<td>100</td>
<td>0</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

Operate with a flow rate of 1.1 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 210 nm. Maintain the column temperature at 30°C.

Prepare the following solutions in mobile phase A.

For solution (1) dissolve about 30 mg of the test substance and dilute to 10 mL. For solution (2) dilute 1.0 mL of solution (1) to 200.0 mL. For solution (3) dilute 2.0 mL of solution (2) to 10.0 mL. For solution (4) dissolve 3.0 mg of clindamycin phosphate for system suitability RS (containing clindamycin phosphate and the impurities B, E, F, G, I, J, K and L) and dilute to 1.0 mL.

Inject 20 μL of solution (4).

Use the chromatogram obtained with solution (4) and the chromatogram supplied with clindamycin phosphate for system suitability RS to identify the peaks due to the impurities B, E, F, G, I, J, K and L. The impurities are eluted at the following relative retention with reference to clindamycin phosphate (retention time about 12 minutes): impurity F about 0.15; impurity G about 0.19; impurity I about 0.34; impurity B about 0.45; impurity L about 0.64; impurity J about 1.20; impurity E about 1.73; and impurity K about 1.90.

The test is not valid unless the resolution between the peaks due to impurity F and the peak due to impurity G is at least 2.0.

Inject alternately 20 μL each of solution (1), (2) and (3).

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to either impurity B or L is not greater than 2 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);
- the area of any peak corresponding to either impurity E or F is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);
- the area of any peak corresponding to either impurity G, I, J or K is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (3) (0.5%);
- the area of any other impurity peak is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.10 %);
- the sum of the areas of all impurities is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (2.0 %). Disregard any peak with an area less
than 0.5 times the area of the principal peak in the chromatogram obtained with solution (3) (0.05%).

**Assay**

Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (15 cm × 4.6 mm) packed with end-capped particles of silica gel, the surface of which has been modified with chemically-bonded octadecysilyl groups (5 µm).

As the mobile phase use a mixture of 21 volumes of acetonitrile for chromatography R and 79 volumes of phosphate buffer pH 6.0. Prepare the phosphate buffer pH 6.0 by dissolving 13.6 g of potassium dihydrogen phosphate R in 750 mL of water R, adjust the pH to 6.0 with potassium hydroxide (~450 g/L) TS and dilute to 1000 mL with water R.

Prepare the following solutions in mobile phase. For solution (1) dissolve about 30 mg of the test substance and dilute to 10 mL. For solution (2) dissolve 30 mg of Clindamycin phosphate and dilute to 10.0 mL. For solution (3) use a solution containing 0.12 mg lincomycin per mL and 0.24 mg of clindamycin phosphate RS per mL.

Operate with a flow rate of 1.1 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 210 nm.

Inject 20 µl of solution (3). In the chromatogram the following peaks are eluted at the following relative retentions with reference to clindamycin phosphate (retention time about 8.0 minutes): lincomycin about 0.32. The assay is not valid unless the resolution between the peaks due to clindamycin phosphate and lincomycin is at least 7.0.

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

Measure the areas corresponding to clindamycin phosphate obtained in the chromatograms from solutions (1) and (2) and calculate the percentage content of clindamycin phosphate (C_{18}H_{34}ClIN_{2}O_{8}PS), using the declared content of clindamycin phosphate (C_{18}H_{34}ClIN_{2}O_{8}PS) in clindamycin phosphate RS.

**Additional requirements for Clindamycin phosphate for parenteral use**

Complies with the monograph for Parenteral preparations.

**Bacterial endotoxins.** If intended for use in the manufacture of a parenteral dosage form without a further appropriate procedure for the removal of bacterial endotoxins, carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.6 IU of endotoxin RS per mg of clindamycin.

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

**Impurities**

A. Methyl 6,8-dideoxy-6-[[[(2S,4R)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-D-erythro-α-D-galacto-octopyranoside (lincomycin) (degradation product)

2 A Symmetry C18 column was found suitable.
B. Methyl 7-chloro-6,7,8-trIDEOxy-6-[[[(2S,4R)-4-ethyl-1-methylpyrrolidin-2-yl]carbonyl]amino]-2-O-phosphono-1-thio-L-threo-α-D-galacto-octopyranoside (clindamycin B-2-phosphate) (synthesis-related impurity)

C. Methyl 7-chloro-6,7,8-trIDEOxy-6-[[[(2S,4R)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-3-O-phosphono-1-thio-L-threo-α-D-galacto-octopyranoside (clindamycin-3-phosphate) (synthesis-related impurity)

D. Methyl 7-chloro-6,7,8-trIDEOxy-6-[[[(2S,4R)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-4-O-phosphono-1-thio-L-threo-α-D-galacto-octopyranoside (clindamycin-4-phosphate) (synthesis-related impurity)

E. Methyl 7-chloro-6,7,8-trIDEOxy-6-[[[(2S,4R)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-L-threo-α-D-galacto-octopyranoside (clindamycin) (synthesis-related impurity / degradation product)
F. Methyl 6,8-dideoxy-6-[[[(2S,4R)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-2-O-phosphono-1-thio-D-erythro-α-D-galacto-octopyranoside (lincomycin 2-phosphate) (degradation product)

G. Methyl 6,8-dideoxy-2,4-O-(hydroxyphosphoryl)-6-[[[(2S,4R)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-D-erythro-α-D-galacto-octopyranoside (2,4-phosphatidyl lincomycin) (synthesis-related impurity)

H. Methyl 7-chloro-6,7,8-trideoxy-6-[[[(2S,4R)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-2,3-di-O-phosphono-1-thio-L-threo-α-D-galacto-octopyranoside (clindamycin-2,3-bisphosphate) (synthesis-related impurity)

I. Methyl 7-chloro-6,7,8-trideoxy-6-[[[(2S,4R)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-2,4-di-O-phosphono-1-thio-L-threo-α-D-galacto-octopyranoside (clindamycin 2,4-bisphosphate)
J. Methyl 7-chloro-6,7,8-trideoxy-6-[[[(2S)-1-methyl-4-propylienedepyrrolidin-2-yl]carbonyl]amino]-2-O-phosphono-1-thio-L-threo-α-D-galacto-octopyranoside (propylidene analog of clindamycin 2-phosphate)

K. 2,2'-Oxybis(hydroxyphosphoryl)bis[methyl 7-chloro-6,7,8-trideoxy-6-[[[(2S,4R)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-L-threo-α-D-galacto-octopyranoside] (diclindamycin pyrophosphate)

L. Methyl 7-chloro-6,7,8-trideoxy-6-[[[(2S,4R)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-2-O-phosphono-1-thio-D-erythro-α-D-galacto-octopyranoside (7-epiclindamycin 2-phosphate) (degradation product)

Reagents to be established

Potassium hydroxide (~450g/L) TS
A solution of potassium hydroxide R containing about 450 g of KOH per litre.
Clindamycin phosphate injection  
*(Clindamycini phosphatis injectio)*

This is a draft proposal of a revised monograph for *The International Pharmacopoeia* (Working document QAS/16.679, August 2016).

The working document is available for comment at [www.who.int/medicines/areas/quality_safety/quality_assurance/projects](http://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.

**Description.** A clear, colourless or almost colourless solution.

**Category.** Antibacterial.

**Storage.** Clindamycin injection should be stored at a temperature not exceeding 30°C. It should not be refrigerated and it should not be allowed to freeze.

**Additional information.** Strength in the current WHO Model List of Essential Medicines (EML): 150 mg (as phosphate) per mL. Strengths in the current EML for Children: 150 mg (as phosphate) per mL.

**Labelling.** The designation of the container should state that the active ingredient is the phosphate form and the quantity should be indicated in terms of equivalent amount of clindamycin.

**Requirements**

Complies with the monograph for *Parenteral preparations*.

**Definition.** Clindamycin phosphate injection is a sterile solution of Clindamycin phosphate in water for injections. It contains not less than 90.0% and not more than 110.0% of the amount of clindamycin C\textsubscript{18}H\textsubscript{33}ClN\textsubscript{2}O\textsubscript{5}S stated on the label.

**Identity tests**

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

A. Carry out the test as described under 1.14.4 **High-performance liquid chromatography** using the conditions given under “Assay”. The retention time of the principle peak in the chromatogram obtained with solution (1) is similar to that in the chromatogram obtained with solution (2).

B. Carry out the test as described under 1.14.1 **Thin-layer chromatography** using silica gel R3 as the coating substance and a mixture of 20 volumes of glacial acetic acid R, 20 volumes of water R and 60 volumes of 1-butanol R as the mobile phase. Apply separately to the plate 5 μL of each of the following 3 solutions in methanol R. For solution (A) dilute a quantity of the injection to obtain a solution containing the equivalent of 2.0 mg of Clindamycin per mL. For solution (B) use clindamycin phosphate RS to obtain a solution containing 2.0 mg of clindamycin phosphate per mL. For solution (C) dissolve 10 mg of lincomycin hydrochloride RS in 5 mL of solution (B). After removing the plate from the chromatographic chamber, allow it to dry at 105°C for 30 minutes. Spray the plate with potassium permanganate (1 g/L) TS and examine the chromatogram in daylight.
The principal spot obtained with solution (A) corresponds in position, appearance and intensity to that obtained with solution (B). The test is not valid unless the chromatogram obtained with solution (C) shows two clearly separated spots.

C. Boil 1 mL of the injection under a reflux condenser with a mixture of 5 mL of sodium hydroxide (~400 g/L) TS and 5 mL of water for 90 minutes. Cool and add 5 mL of nitric acid (~1000 g/L) TS. Extract with three 15 mL quantities of dichloromethane R and discard the extracts. Filter the upper aqueous layer through a paper filter; the filtrate yields reaction B described under 2.1 General identification tests as characteristic of orthophosphates.

**pH value (1.13).** pH of the injection, 5.5–7.0.

**Related substances**

Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (15 cm × 4.6 mm) packed with end-capped particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 µm).\(^1\)

Use the following conditions for gradient elution:

- mobile phase A: 21 volumes of acetonitrile for chromatography R and 79 volumes of phosphate buffer pH 6.0;
- mobile phase B: 60 volumes of acetonitrile for chromatography R and 40 volumes of phosphate buffer pH 6.0.

Prepare the phosphate buffer pH 6.0 by dissolving 13.6 g of potassium dihydrogen phosphate R in 750 mL of water R, adjust the pH to 6.0 with potassium hydroxide (~450 g/L) TS and dilute to 1000 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>0–13</td>
<td>100</td>
<td>0</td>
<td>Isocratic</td>
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<td>13–18</td>
<td>100 to 50</td>
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<td>Linear gradient</td>
</tr>
<tr>
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<td>50</td>
<td>50</td>
<td>Isocratic</td>
</tr>
<tr>
<td>39–40</td>
<td>50 to 100</td>
<td>50 to 0</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>40–55</td>
<td>100</td>
<td>0</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

Operate with a flow rate of 1.1 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 210 nm. Maintain the column temperature at 30°C.

Prepare the following solutions in mobile phase A.

For solution (1) dilute 2.0 mL of the injection to 100.0 mL. For solution (2) dilute 1.0 mL of solution (1) to 100.0 mL. For solution (3) use a solution containing 0.12 mg lincomycin hydrochloride RS per mL, 0.24 mg of clindamycin phosphate RS per mL and 15 µg of benzyl alcohol R per mL.

Inject 20 µL of solution (3). The test is not valid unless in the chromatogram obtained with solution (3), the resolution factor between the peaks due to lincomycin and clindamycin phosphate is at least 7.7. The following peaks are eluted at the following relative retentions with reference to clindamycin phosphate (retention time about 12 minutes): lincomycin: about x; benzyl alcohol: about 0.8. [to be added]

Inject alternately 20 µL each of solution (1) and (2).

\(^1\) A Symmetry C18 column was found suitable.
In the chromatogram obtained with solution (1):

- the area of any impurity peak is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (3) (3.0%);
- the sum of the areas of all impurities is not greater than 8 times the area of the principal peak in the chromatogram obtained with solution (2) (8.0%). Disregard any peak due to benzyl alcohol, if present, and any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

**Assay**

Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (15 cm × 4.6 mm) packed with end-capped particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 µm).²

As the mobile phase use a mixture of 21 volumes of acetonitrile for chromatography R and 79 volumes of phosphate buffer pH 6.0. Prepare the phosphate buffer pH 6.0 by dissolving 13.6 g of potassium dihydrogen phosphate R in 750 mL of water R, adjust the pH to 6.0 with potassium hydroxide (~450 g/L) TS and dilute to 1000 mL with water R.

Prepare the following solutions in mobile phase. For solution (1) dilute 1.0 mL of the injection to 100.0 mL. For solution (2) dissolve 36 mg of clindamycin phosphate RS and dilute to 20.0 mL. For solution (3) use a solution containing 0.12 mg lincomycin per mL, 0.24 mg of clindamycin phosphate RS per mL and 15 µg of benzyl alcohol R per mL.

Operate with a flow rate of 1.1 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 210 nm.

Inject 20 µL of solution (3). In the chromatogram the following peaks are eluted at the following relative retentions with reference to clindamycin phosphate (retention time about 8.0 minutes): benzyl alcohol about 0.6. The assay is not valid unless the resolution between the peaks due to clindamycin phosphate and benzyl alcohol is at least 3.0 and the resolution between the peaks due to clindamycin phosphate and lincomycin is at least 7.0.

Inject alternately 20 µL each of solutions (1) and (2). Measure the areas of the peaks corresponding to clindamycin phosphate obtained in the chromatograms from solutions (1) and (2) and calculate the percentage content of clindamycin (C₁₈H₃₄ClN₂O₅S) in the injection using the declared content of C₁₈H₃₄ClN₂O₅PS in clindamycin phosphate RS. Each mg of clindamycin phosphate (C₁₈H₃₄ClN₂O₅PS) is equivalent to 0.8416 mg of clindamycin (C₁₈H₃₃ClN₂O₅S).

**Bacterial endotoxins.** Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.10 IU of endotoxin RS per mg of clindamycin.

**Impurities**

The impurities limited by the requirements of this monograph include those listed in the monograph on Clindamycin phosphate.

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² A Symmetry C18 column was found suitable.
Ceftiraxone sodium

*(Ceftiraxonomum natricum)*

This is a revised draft proposal of a monograph for *The International Pharmacopoeia* (Working document QAS/15.644/Rev.1, July 2016).

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: schmidtth@who.int.

C\textsubscript{18}H\textsubscript{16}N\textsubscript{8}Na\textsubscript{2}O\textsubscript{7}S\textsubscript{3}·3½H\textsubscript{2}O

**Relative molecular mass.** 661.60

Chemical name. Disodium (6\textsubscript{R},7\textsubscript{R})-7-[[((2Z)-(2-aminothiazol-4-yl)(methoxyimino) acetyl]amino]-3-[[((2-methyl-6-oxido-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)sulfanyl]meth- yl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate hemiheptahydrate. CAS Reg. No. 104376-79-6.

**Description.** Almost white or yellowish, slightly hygroscopic, crystalline powder.

**Solubility.** Freely soluble in water, sparingly soluble in methanol, very slightly soluble in anhydrous ethanol.

**Labelling.** The label states, where applicable:

- that the substance is free of bacterial endotoxins;
- that the substance is sterile.

**Category.** Antibacterial

**Storage.** Ceftriaxone sodium should be kept in an air-tight container protected from light. If the substance is sterile, store in a sterile and air-tight container protected from light.

**Manufacture.** Where necessary, the production method is validated to demonstrate that the substance, if tested, would comply with limits of not more than 20 ppm for N,N-dimethylaniline and 0.8% for 2-ethylhexanoic acid.

**Additional information.** Ceftriaxone sodium is a semi-synthetic product derived from a fermentation product.

**Requirements**

Ceftriaxone sodium contains not less than 96.0% and not more than 102.0% of C\textsubscript{18}H\textsubscript{16}N\textsubscript{8}Na\textsubscript{2}O\textsubscript{7}S\textsubscript{3}, calculated with reference to the anhydrous substance.
Identity tests

- Either tests A and C 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 ceftriaxone sodium RS or with the reference spectrum of ceftriaxone sodium.

B. Carry out the test as described under **1.14.4 High-performance liquid chromatography** using the conditions given under the Assay. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time of the peak corresponding to ceftriaxone in the chromatogram obtained with solution (2).

C. When tested for sodium as described under **2.1 General identification tests**, yields the characteristic reaction.

**Specific optical rotation (1.4)**. Dissolve 0.250 g in water R and dilute to 25.0 mL with the same solvent. Calculate with reference to the anhydrous substance; \( [\alpha]_{D}^{20^\circ} = -155^\circ \) to \(-170^\circ\).

**Clarity and colour of solution**. Dissolve 2.40 g in carbon-dioxide-free water R and dilute to 20.0 mL with the same solvent (Solution A). Dilute 2 mL of Solution A to 20 mL carbon-dioxide-free water R. The solution is clear and not more intensely coloured than reference solution \( Y_5 \) or \( BY_5 \) when compared as described under **1.11.2 Degree of coloration of liquids**. (Keep the remaining solution (Solution A) for the “pH value”.)

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

**pH value (1.13)**. pH of the solution prepared for the “Clarity and colour of solution” (Solution A), 6.0 to 8.0.

**Water**. Determine as described under **2.8 Determination of water by the Karl Fischer method**, method A, using 0.100 g of the test substance. The water content is not less than 80 mg per g and not more than 110 mg per g.

**Bacterial endotoxins**. If intended for use in the manufacture of a parenteral dosage form without a further appropriate procedure for the removal of bacterial endotoxins, carry out the test as described under **3.4 Test for bacterial endotoxins**; contains not more than 0.20 IU of endotoxin per mg of ceftriaxone sodium.

**Related substances**

Carry out the test as described under **1.14.4 High-performance liquid chromatography** using the conditions given below under assay method.

Prepare the following solutions in mobile phase: for solution (1) dissolve about 30 mg of the test substance and dilute to 100.0 mL. For solution (2) dilute 1 volume of solution (1) to 100 volumes. For solution (3) dissolve about 5 mg ceftriaxone sodium RS and 5 mg of ceftriaxone impurity A to 100.0 mL.

Inject 20 µL of solution (3). The test is not valid unless the resolution factor between the peaks due to ceftriaxone and ceftriaxone impurity A is at least 3.0. Ceftriaxone impurity A is eluted at a relative retention of about 1.4 with reference to ceftriaxone (retention time about 9 min).

Inject alternately 20 µL each of solution (1) and (2). Record the chromatograms for about 2 times the retention time of ceftriaxone.
In the chromatogram obtained with solution (1):

- the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0 %);
- the sum of the areas of all peaks, other than the principal peak, is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5 %). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1 %).

**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 particles of base-deactivated silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 µm).

As the mobile phase use a solution prepared as follows: dissolve 2.0 g of tetradecylammonium bromide R and 2.0 g of tetraheptylammonium bromide R in a mixture of 440 mL of water R, 55 mL of phosphate buffer pH 7.0 (0.067 mol/L) TS, 5.0 mL of citrate buffer pH 5.0 TS and 500 mL of acetonitrile R and filter.

Prepare the following solutions in mobile phase. For solution (1) dissolve 30 mg of the test substance, accurately weighed and dilute to 100.0 mL. For solution (2) dissolve about 30 mg of ceftriaxone sodium RS, accurately weighed and dilute to 100.0 mL. For solution (3) dissolve about 5 mg ceftriaxone sodium RS and about 5 mg of ceftriaxone impurity A and dilute to 100.0 mL.

Operate with a flow rate of 1.5 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 254 nm.

Inject 20 µL of solution (3). The test is not valid unless the resolution factor between the peaks due to ceftriaxone and ceftriaxone impurity A is at least 3.0.

Inject alternately 20 µL each of solution (1) and (2). Measure the areas of the peaks corresponding to ceftriaxone and calculate the percentage content of C_{18}H_{16}N_{8}Na_{2}O_{7}S_{3}, using the declared content of C_{18}H_{16}N_{8}Na_{2}O_{7}S_{3} in ceftriaxone sodium RS.

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

**Impurities**

[Note from the Secretariat. The structures of the impurities will be added at a later stage.]

A. (6R,7R)-7-[(E)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[[[(6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)thio][methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (Ceftriaxone E-isomer)

B. (Z)-2-(2-Aminothiazol-4-yl)-N-[[5aR,6R]-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-b]furo[3,4-d][1,3]thiazin-6-yl]-2-(methoxyimino)acetamide. (Deacetylcefotaxime lactone)

C. (6R,7R)-7-Amino-3-[[[(6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)thio][methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (Deacyl ceftriaxone)

D. (Z)-5-Benzothiazol-2-yl 2-(2-aminothiazol-4-yl)-2-(methoxyimino)thioacetate (Ceftriaxone benzothiazolyl oxime)

---

1 Hypersil BDS C18 has been found suitable.

F. 3-Mercapto-2-methyl-1,2-dihydro-1,2,4-triazine-5,6-dione. (Ceftriaxone triazine analog)

G. (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-{[(6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)thio]methyl}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid (Ceftriaxone 3-ene isomer).

Reagents to be included

Citrate buffer, pH 5 TS

Procedure. Dissolve 20.17 g of citric acid R in 800 ml of water R, adjust to pH 5.0 with sodium hydroxide (~400 g/L) TS and dilute to 1000 mL with water R.

Tetradecylammonium bromide R

C_{40}H_{84}BrN. Chromatographic reagent grade of commerce.

Description. White to almost white crystals, or a crystalline powder.

Melting point. 88-89°C.

Tetraheptylammonium bromide R

C_{28}H_{60}BrN. Chromatographic reagent grade of commerce.

Description. White, flaky powder.

Melting range. Between 89-91°C.

***
This is a revised draft proposal of a monograph for *The International Pharmacopoeia* (Working document QAS/15.645/Rev.1, July 2016).

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

**Description.** A white to almost white powder.

**Category.** Antibacterial.

**Storage.** Ceftriaxone for injection should be stored in a tightly closed container. The reconstituted solution should be used immediately after preparation.

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

**Additional information.** Strengths in the current WHO Model List of Essential Medicines (EML): 250 mg, 1 g (as sodium salt) in vial. Strength in the current WHO EML for children: 250 mg, 1 g (as sodium salt) in vial.

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

**Requirements**

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

**Definition.** Ceftriaxone for injection is a sterile powder containing Ceftriaxone sodium with or without excipients.

Ceftriaxone for Injection contains not less than 90.0% and not more than 110.0% of the labelled amount of ceftriaxone \((C_{18}H_{18}N_{8}O_{7}S_{3})\).

**Identity tests**

- Either tests A and C 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 ceftriaxone sodium RS or with the reference spectrum of ceftriaxone sodium.

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

C. When tested for sodium as described under 2.1 General identification tests, yields the characteristic reaction.
**Water.** Determine as described under 2.8 Determination of water by the Karl Fischer method, method A, using about 0.100 g of the powder. The water content is not more than 110 mg per g.

**Clarity and colour of solution.** Dissolve 2.40 g in carbon-dioxide-free water R and dilute to 20.0 mL with the same solvent (Solution A). Dilute 2 mL of Solution A to 20 mL carbon-dioxide-free water R. The solution is clear and not more intensely coloured than reference solution YW2 when compared as described under 1.11 Colour of liquids. (Keep the remaining solution (Solution A) for the “pH value”.)

**pH value (1.13).** pH of the solution prepared for the “Clarity and colour of solution” (Solution A), 6.0 to 8.0.

**Bacterial endotoxins.** Carry out the test described under 3.4 Test for bacterial endotoxins, contains not more than 0.20 IU of endotoxin per mg of ceftriaxone.

**Related substances**

Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given below under assay method.

Prepare the following solutions in mobile phase: for solution (1) dissolve about 30 mg of the powder and dilute to 100.0 mL. For solution (2) dilute 1 volume of solution (1) to 100 volumes. For solution (3) dissolve about 5 mg ceftriaxone sodium RS and 5 mg of ceftriaxone impurity A to 100.0 mL.

Inject 20 µL of solution (3). The test is not valid unless the resolution factor between the peaks due to ceftriaxone and ceftriaxone impurity A is at least 3.0. Ceftriaxone impurity A is eluted at a relative retention of about 1.4 with reference to ceftriaxone (retention time about 9 min).

Inject alternately 20 µL each of solutions (1) and (2). Record the chromatograms for about 2 times the retention time of ceftriaxone.

In the chromatogram obtained with solution (1):

- the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);
- the sum of the areas of all peaks, other than the principal peak, is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

**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 particles of base-deactivated silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 µm).²

As the mobile phase use a solution prepared as follows: dissolve 2.0 g of tetradeclammonium bromide R and 2.0 g of tetradehpylammonium bromide R in a mixture of 440 mL of water R, 55 mL of phosphate buffer, pH 7.0 (0.067 mol/L) TS, 5.0 mL of a citrate buffer pH 5.0 TS and 500 mL of acetonitrile R and filter.

Prepare the following solutions in mobile phase: for solution (1) determine the weight of the contents of 10 containers. Transfer a quantity of the mixed contents containing about 30 mg of ceftriaxone, accurately weighed, to a 100 mL volumetric flask, dissolve and dilute to volume.

² Hypersil BDS C18 has been found suitable.
For solution (2) dissolve about 35 mg of ceftriaxone sodium RS, accurately weighed and dilute to 100.0 mL. For solution (3) dissolve about 5 mg ceftriaxone sodium RS and 5 mg of ceftriaxone impurity A to 100.0 mL.

Operate with a flow rate of 1.5 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 254 nm.

Inject 20 µL of solution (3). The test is not valid unless the resolution factor between the peaks due to ceftriaxone and ceftriaxone impurity A is at least 3.0.

Inject 20 µL of solution (1) and (2) Measure the areas of the peaks corresponding to ceftriaxone and calculate the content of ceftriaxone (C\textsubscript{18}H\textsubscript{16}N\textsubscript{8}O\textsubscript{7}S\textsubscript{3}) per container, using the declared content of C\textsubscript{18}H\textsubscript{16}N\textsubscript{8}Na\textsubscript{2}O\textsubscript{7}S\textsubscript{3} in ceftriaxone sodium RS. Each mg of C\textsubscript{18}H\textsubscript{16}N\textsubscript{8}Na\textsubscript{2}O\textsubscript{7}S\textsubscript{3} is equivalent to 0.9274 mg of C\textsubscript{18}H\textsubscript{16}N\textsubscript{8}O\textsubscript{7}S\textsubscript{3}.

**Impurities**

The impurities limited by the requirements of this monograph include those listed in the monograph for Ceftriaxone sodium.
Mebendazole
(Mebendazolum)

This is a draft proposal of a revised monograph for The International Pharmacopoeia (Working document QAS/16.674, July 2016).

The working document with line numbers and tracked changes 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.

[Note from the Secretariat. It is proposed to revise the monograph on Mebendazole of The International Pharmacopoeia.]

[Note from the editor. In accordance with WHO editorial policy the text reproduced below does not include tracked changes. Changes from the current monograph are indicated by insert and delete in the working document available at the above-mentioned web address.]

\[C_{16}H_{13}N_{3}O_{3}\]

Relative molecular mass. 295.3


Description. A white or almost white powder.

Solubility. Practically insoluble in water, dilute mineral acids and ethanol (~750 g/L) TS; freely soluble in formic acid (~1080 g/L) TS.

Category. Anthelmintic.

Storage. Mebendazole should be kept in a well-closed container, protected from light.

Additional information. Mebendazole exhibits polymorphism.

Requirements

Definition. Mebendazole is polymorph C, the crystal form of mebendazole RS. Mebendazole contains not less than 99.0% and not more than 101.0% of mebendazole (\(C_{16}H_{13}N_{3}O_{3}\)), calculated with reference to the dried substance.
Identity test

Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum obtained from the solid state is concordant with the spectrum obtained from mebendazole RS (confirmation of polymorphic form C).

**Heavy metals.** Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 μg/g.

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

**Loss on drying.** Dry at 105°C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 4 hours; 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 (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:

- **mobile phase A:** 7.5 g/L solution of ammonium acetate R;
- **mobile phase B:** Acetonitrile R.

<table>
<thead>
<tr>
<th>Time (minutes)</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.

Prepare the following solutions in dimethylformamide R. For solution (1) dissolve 25.0 mg of the test substance and dilute to 25.0 mL. For solution (2) dissolve 1.0 mL of solution (1) to 100.0 mL. Dilute 5.0 mL of this solution to 20.0 mL. For solution (3) dissolve 5.0 mg of mebendazole for system suitability RS (containing the impurities A, B, C, D, E, F and G) and dilute to 5.0 mL.

Inject 10 μL of solution (3). The test is not valid unless the peak-to-valley ratio (Hp/Hv) is at least 4, where Hp is the height above the baseline of the peak due to impurity D (relative retention about 1.1) and Hv is the height above the baseline of the lowest point of the curve separating this peak from the peak due to mebendazole (retention time about 12 minutes).

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

Use the chromatogram obtained with solution (3) and the chromatogram supplied with mebendazole for system suitability RS to identify the peaks due to the impurities A, B, C, D, E, F and G. The impurities are eluted at the following relative retention with reference to mebendazole (retention time about 12 minutes): impurity A about 0.4; impurity B about 0.5;

¹ A HYPERSIL BDS C₁₈ column has been found suitable.
impurity C about 0.7; impurity D about 1.1; impurity E about 1.3; impurity F about 1.4 and impurity G about 1.6.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity A, impurity B, impurity C, impurity D, impurity E or impurity F is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.25%);
- the area of any peak corresponding to impurity G, when multiplied by a correction factor of 1.4, is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);
- the area of any other impurity peak is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.10%);
- the sum of the areas of all impurities is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%). Disregard any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

**Assay.** Dissolve about 0.250 g, accurately weighed, in 3 mL of anhydrous formic acid R and add 50 mL of a mixture of 1 volume of anhydrous acetic acid R and 7 volumes of methyl ethyl ketone R. Titrate with perchloric acid (0.1 mol/L) VS, determining the end-point potentiometrically as described under **2.6 Non-aqueous titration**.

Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 29.53 mg of C_{16}H_{13}N_{3}O_{3}.

**Impurities**

A. (2-Amino-1H-benzimidazol-5-yl)phenylmethanone

B. (2-Hydroxy-1H-benzimidazol-5-yl)phenylmethanone

C. (2-Amino-1-methyl-1H-benzimidazol-5-yl)phenylmethanone
D. Methyl (5-benzoyl-1-methyl-1H-benzimidazol-2-yl)carbamate

E. Ethyl (5-benzoyl-1H-benzimidazol-2-yl)carbamate

F. Methyl [5-(4-methylbenzoyl)-1H-benzimidazol-2-yl]carbamate

G. N,N′-bis(5-benzoyl-1H-benzimidazol-2-yl)urea

***
Mebendazole chewable tablets
(Mebendazoli compressi manducabili)

This is a draft proposal of a revised monograph for The International Pharmacopoeia (Working document QAS/16.661, July 2016).

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.

[Note from the Secretariat. The draft revision of the monograph is based on samples received in response to a letter sent out to pharmaceutical manufacturers in November 2014, inviting their collaborating in the development of this document. Manufacturers that have not yet donated samples and/or specifications are again kindly invited to do so. For more information, kindly contact Dr Herbert Schmidt at schmidth@who.int.]

Category. Anthelminthic.

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

Labelling. The designation on the container should state that the tablets may be chewed, swallowed whole, crushed and mixed with food or liquid or dispersed in water.

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 chewable tablets contain Mebendazole in a suitable basis that may contain suitable flavouring agents. Mebendazole chewable tablets contain not less than 90.0% and not more than 110.0% of the amount of mebendazole (C_{16}\text{H}_{13}\text{N}_{3}\text{O}_{3}) stated on the label.

Manufacture. The formulation, manufacturing process and product packaging of Mebendazole chewable 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, B and C or tests A, B and D 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. Shake a quantity of the powdered tablets containing 0.04 g of Mebendazole with 2 mL of sodium hydroxide (~80 g/L) TS and heat the yellowish coloured suspension; the solution is yellow. Add a few drops of copper (II) sulfate (160 g/L) TS; a greenish precipitate is produced. Add a few drops of ammonia (~100 g/L) TS; the colour of the precipitate turns to greenish blue.

C. 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 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 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:

- **mobile phase A:** 7.5 g/L solution of ammonium acetate R;
- **mobile phase B:** Acetonitrile R.

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<tr>
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<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>
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<tr>
<td>26–36</td>
<td>80</td>
<td>20</td>
<td>Isocratic re-equilibration</td>
</tr>
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</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.

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 the solvent mixture, mix and filter. For solution (2) dilute 1.0 mL of solution (1) to 100.0 mL with the solvent mixture.

\(^1\) A HYPERSIL BDS C\(_{18}\) column has been found suitable.
Dilute 5.0 mL of this solution to 20.0 mL with the solvent mixture. For solution (3) transfer 10 mg mebendazole R 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 (C₁₆H₁₃N₃O₃) 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 (C₁₆H₁₃N₃O₃) in the medium using the declared content of (C₁₆H₁₃N₃O₃) 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 (C₁₆H₁₃N₃O₃) 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 (C₁₆H₁₃N₃O₃) in the medium using the declared content of (C₁₆H₁₃N₃O₃) in mebendazole RS. The amount in solution for each tablet is not less than 75% (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 x 4.6 mm) packed with octadecylsilyl base-deactivated silica gel for chromatography R (3 µm).2

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 (C₁₆H₁₃N₃O₃) in the chewable tablets using the declared content of C₁₆H₁₃N₃O₃ in mebendazole RS.

Impurities

The impurities limited by the requirements of this monograph includes impurity A listed in the monograph for Mebendazole.

Reagents to be established

**Mebendazole R**

Mebendazole of a suitable quality should be used.

**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.

---

2 A HYPERSIL BDS C18 column has been found suitable.
Methylthioninium chloride
(*Methylthioninii chloridum*)

This is a draft proposal of a monograph for *The International Pharmacopoeia* (Working document QAS/16.675, July 2016).

The working document with line numbers is available for comment at [www.who.int/medicines/areas/quality_safety/quality_assurance/projects](http://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: schmidt@who.int.

[Note from the Secretariat. It is proposed to revise the monograph on Methylthioninium chloride.]

[Note from the editor. In accordance with WHO editorial policy the text reproduced below does not include tracked changes. Changes from the current monograph are indicated by insert and delete in the working document available at the above-mentioned web address.]

**Molecular formula.** $C_{16}H_{18}ClN_{3}S$ (anhydrous); $C_{16}H_{18}ClN_{3}S.H_{2}O$ (monohydrate); $C_{16}H_{18}ClN_{3}S.3H_{2}O$ (trihydrate); $C_{16}H_{18}ClN_{3}S.5H_{2}O$ (pentahydrate).

**Relative molecular mass.** 319.9 (anhydrous); 337.9 (monohydrate); 373.9 (trihydrate); 409.9 (pentahydrate).

**Graphic formula**

n=0 (anhydrous)  
 n=1 (monohydrate)  
 n=3 (trihydrate)  
 n=5 (pentahydrate)

**Chemical name.** C.I. Basic Blue 9; 3,7-bis(dimethylamino)phenothiazin-5-ium chloride; CAS Reg. No. 61-73-4 (anhydrous).

C.I. Basic Blue 9 monohydrate; 3,7-bis(dimethylamino)phenothiazin-5-ium chloride monohydrate; CAS Reg. No. 122965-43-9 (monohydrate).

C.I. Basic Blue 9 trihydrate; 3,7-bis(dimethylamino)phenothiazin-5-ium chloride trihydrate; CAS Reg. No. 7220-79-3 (trihydrate).

C.I. Basic Blue 9 pentahydrate; 3,7-bis(dimethylamino)phenothiazin-5-ium chloride pentahydrate; CAS Reg. No. 32680-41-4 (pentahydrate).

**Other name.** Methylene blue

**Description.** Dark green crystals with a metallic lustre or a dark green, crystalline powder.

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

Storage. Methylthioninium chloride should be kept in a tightly closed container, protected from light, at a temperature not exceeding 30°C.

Additional information. Methylthioninium chloride is hygroscopic.

Requirements

Definition. Methylthioninium chloride contains not less than 93.0% and not more than 102.0% (“Assay”, method A) or not less than 98.0% and not more than 102.0% (“Assay”, method B) of C_{10}H_{18}ClN_{3}S, calculated with reference to the dried substance.

Identity tests

• Either tests A and F or any two of tests B, C, D or E together with test F may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from methylthioninium chloride RS or with the reference spectrum of methylthioninium chloride.

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

C. The absorption spectrum (1.6) of a 5 μg/mL solution in hydrochloric acid (~70 g/L) TS, when observed between 230 nm and 800 nm, exhibits 4 maxima at about 258 nm, 288 nm, 680 nm, and 745 nm.

D. 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 3 volumes of acetic acid R, 3 volumes of ethanol R and 4 volumes of water R as the mobile phase. Apply separately to the plate 2 μL of each of the following 2 solutions in methanol R containing (A) 0.1 mg of the test substance per mL and (B) 0.1 mg of methylthioninium chloride RS per mL. After removing the plate from the chromatographic chamber allow it to dry in air or in a current of cool air. Examine the chromatogram in daylight.

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

E. Dissolve 1 mg in 10 mL of water R; a deep blue color is produced. Add 2.0 mL of hydrochloric acid (~70 g/L) TS and 0.25 g of zinc R powder; the color of the solution is discharged. Filter and expose the filtrate to the air; the blue color of the solution reappears.

F. Mix 0.05 g of the substance to be investigated with 0.5 g of anhydrous sodium carbonate R in a porcelain crucible. Carefully heat the mixture to a red glow for 10 minutes. Cool, dissolve the residue in 10 mL of nitric acid (~130 g/L) TS and filter. The filtrate yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Copper or zinc. Prepare the following solutions. For solution (1) ignite 1.0 g in a porcelain crucible using as low a temperature as practicable, until all of the carbon is oxidized. Cool the residue, add 15 mL of nitric acid (~130 g/L) TS and boil for 5 minutes. For solution (2) boil a quantity of copper(II) sulfate R, equivalent to 200 μg of Cu, with 15 mL of nitric acid (~130 g/L) TS for 5 minutes. Filter separately the cooled solutions (1) and (2) and wash any residue with 10 mL of water. Combine the filtrate and washings of the solution (1) and similarly combine the filtrate and washings of the solution (2); add to each an excess of ammonia (~100 g/L) TS.
and filter the solutions into 50 mL volumetric flasks. Wash the precipitates with small portions of water, adding the washings to the filtrates; dilute the contents of each flask with water to volume, mixing thoroughly. To 25 mL of each of the solutions add 10 mL of hydrogen sulfide TS; no turbidity is produced within 5 minutes (absence of zinc) and any dark colour produced in solution (1) is not more intense than that of solution (2) (the copper content is not more than 0.20 mg/g).

**Iron.** Mix 4 g with 200 mL of water R in a long-necked, round-bottomed flask, add 15 mL of nitric acid (~1000 g/L) TS, heat carefully to boiling and continue boiling until the volume of liquid is reduced to about 20 mL. Allow to cool, add 10 mL of sulfuric acid (~1760 g/L) TS and mix. Heat to boiling and add small successive quantities of nitric acid (~1000 g/L) TS, cooling before each addition, until a colourless liquid is obtained. Heat until white fumes are evolved; if darkening occurs at this stage continue the treatment with nitric acid (~1000 g/L) TS. Finally heat until white fumes are again evolved. Allow the colourless liquid to cool, add 25 mL of a saturated solution of ammonium oxalate R in water, and boil until the slight froth completely subsides. Cool, dilute to 50 mL with water; 5 mL of the diluted solution complies with the 2.2.4 Limit test for iron; not more than 0.10 mg/g.

**Sulfated ash.** Not more than 2.5 mg/g.

**Loss on drying.** Dry at 105°C for 5 hours; it loses not more than 240 mg/g. (The dried substance may be used to produce solution (4) of the test “Related substances”.

**Related substances**

Carry out test as described under 1.14.4 High-performance liquid chromatography using the chromatographic conditions as described under “Assay”, method A.

Prepare the following solutions using as the diluent a mixture of 70 volumes of a 0.1% (v/v) solution of trifluoroacetic acid R (mobile phase A) and 30 volumes of acetonitrile R (mobile phase B).

For solution (1) dissolve about 50 mg of the substance to be examined and dilute to 50.0 mL. Sonicate for 5 minutes. For solution (2) dilute 1.0 mL of solution (1) to 100.0 mL. For solution (3) dilute 5.0 mL of solution (2) to 50.0 mL. For solution (4) dissolve 2.5 mg methylthioninium chloride impurity A RS and dilute to 10.0 mL. Transfer 1.0 mL of this solution to a 10 mL volumetric flask and make up to volume with solution (1). Alternatively, dry the substance to be examined at 105°C for 5 h (the dried substance of the test “Loss on drying” may be used), dissolve 100 mg of the dried substance and dilute to 100.0 mL. Sonicate for 5 minutes.

Inject alternately 5 µL each of solutions (1), (2), (3), (4).

Use the chromatograms obtained with solution (4) and solution (1) to identify the peak due to impurity A. Impurity A is eluted at the relative retention of about 0.8 with reference to methylthioninium (retention time about 11 minutes). The test is not valid unless the resolution between the peaks corresponding to methylthioninium and impurity A is at least 3.5.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity A is not greater than 5 times the area of the principal peak obtained with solution (2) (5.0%);
- the area of any other impurity peak is not greater than the area of the principal peak obtained with solution (3) (0.10%);
- the sum of the areas of all impurity peaks, other than the peak corresponding to impurity A, is not greater than 5 times the area of the principal peak obtained with solution (3) (0.5 %). Disregard any peak with an area less than 0.5 times the area of the principal peak obtained with solution (3) (0.05%).
Assay

- Either method A or B may be applied.

A. Carry out test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (10 cm x 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded phenylsilyl groups (3.5 µm).1

Use the following conditions for gradient elution:

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–5</td>
<td>80</td>
<td>20</td>
<td>Isocratic</td>
</tr>
<tr>
<td>5–25</td>
<td>80 to 30</td>
<td>20 to 70</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>25–32</td>
<td>30</td>
<td>70</td>
<td>Isocratic</td>
</tr>
<tr>
<td>32–35</td>
<td>30 to 80</td>
<td>70 to 20</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>35–40</td>
<td>80</td>
<td>20</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

Operate with a flow of 1.0 mL/min. As a detector use an ultraviolet spectrophotometer set at a wavelength of 246 nm. Maintain the column temperature at 30°C.

Prepare the following solutions using as diluent a mixture of 30 volumes of acetonitrile R and 70 volumes of mobile phase A. For solution (1) dissolve about 50 mg of the substance to be examined, accurately weighed, and dilute to 50.0 mL. Sonicate for 5 minutes. For solution (2) dissolve 50.0 mg of methylthioninium chloride RS and dilute to 50.0 mL. Sonicate for 5 min.

Inject alternately 5 µL each of solutions (1) and (2). The test is not valid unless the symmetry factor of methylthioninium is not more than 3.0.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2) and calculate the percentage content of methylthioninium chloride (C_{16}H_{18}ClN_{3}S), using the declared content of C_{16}H_{18}ClN_{3}S in methylthioninium chloride RS.

B. Dissolve about 100 mg, accurately weighed, in sufficient ethanol (~457 g/L) TS to produce 250.0 mL. Dilute 5.0 mL of this solution to 100.0 mL with ethanol (~457 g/L) TS. Dilute 5.0 mL of this solution to 50.0 mL with ethanol (~457 g/L) TS. Measure the absorbance (1.6) of a 1 cm layer of the diluted solution at the maximum at about 664 nm and calculate the percentage content of methylthioninium chloride (C_{16}H_{18}ClN_{3}S) using the absorptivity value of 2950 methylthioninium chloride.

[Note from the Secretariat: The absorptivity value is so far based on a single determination. It is intended to perform further independent determinations to confirm the value.]

Additional requirements for Methylthioninium chloride for parenteral use

Complies with the monograph for Parenteral preparations.

Bacterial endotoxins. If intended for use in the manufacture of a parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins, carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 2.5 IU of endotoxin RS per mg.

1 An X-Bridge Phenyl column and a Phenomenex Luna 3 µm Phenyl-Hexyl column were found suitable.
Impurities

A. 3-(Dimethylamino)-7-(methylamino)phenothiazin-5-iium chloride (azure B).

B. 3-Amino-7-(dimethylamino)phenothiazin-5-iium (azure A)

C. 3-amino-7-(methylamino)phenothiazin-5-iium (azure C)
Methylthioninium injection
*(Methylthioninii injectio)*

This is a draft proposal of a monograph for *The International Pharmacopoeia* (Working document QAS/16.676, July 2016).

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

**[Note from the Secretariat. The monograph is proposed for inclusion in The International Pharmacopoeia.]**

**Description.** A clear, dark blue solution.

**Category.** Antidote

**Storage.** Store at room temperature, protected from light.

**Additional information.** Strength in the current WHO Model List of Essential Medicines (EML): 10 mg/mL in 10 mL ampoule; other available strength: 5 mg/mL.

**Requirements**

Complies with the monograph for *Parenteral preparations*.

**Definition.** Methylthioninium injection is a sterile solution of Methylthioninium chloride in water for injection. It contains not less than 90.0% and not more than 110.0% of the amount of C₁₆H₁₈ClN₃S stated on the label.

**Identity tests**

- Any two of tests A, B and C may be applied.

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

B. Carry out test as described under 1.14.1 *Thin-layer chromatography* using silica gel R₆ as the coating substance and a mixture of 3 volumes of acetic acid R, 3 volumes of ethanol R and 4 volumes of water R as the mobile phase. Apply separately to the plate 1 µL of each of the following 2 solutions: For solution (A) dilute 1.0 mL of the injection to 20.0 mL with methanol R to obtain a solution with a concentration of 0.5 mg of the methylthioninium chloride per mL. For solution (B) dissolve 10.0 mg of methylthioninium chloride RS and dilute to 20.0 mL with a mixture of water R and methanol R (20:80 v/v). After removing the plate from the chromatographic chamber allow it to dry in air or in a current of cool air. Examine the chromatogram in daylight.

The principal spot obtained with solution (A) corresponds in position, appearance and intensity to that obtained with solution (B).
C. The absorption spectrum (1.6) of a 5 μg/mL solution in hydrochloric acid (~70 g/L) TS, when observed between 230 nm and 800 nm, exhibits 4 maxima at about 258 nm, 288 nm, 680 nm and 745 nm.

pH value (1.13). pH of the injection, 3.0–4.5

**Related substances**

Carry out test as described under 1.14.4 High-performance liquid chromatography using the chromatographic conditions as described under “Assay”, method A.

Prepare the following solutions using as the diluent a mixture of 70 volumes of a 0.1% (v/v) solution of trifluoroacetic acid R (mobile phase A) and 30 volumes of acetonitrile R (mobile phase B).

For solution (1) dilute 1.0 mL of the injection to 20.0 mL to obtain a solution with a concentration of 0.5 mg of the methylthioninium chloride per mL. For solution (2) dilute 1.0 mL of solution (1) to 100.0 mL. For solution (3) dilute 5.0 mL of solution (2) to 50.0 mL. For solution (4) dissolve 2.5 mg methylthioninium chloride impurity A RS and dilute to 10.0 mL. Transfer 1.0 mL of this solution to a 10 mL volumetric flask and make up to volume with solution (1).

Alternatively, dry 100 mg of methylthioninium chloride R at 105°C for 5 hours, dissolve 50 mg of the dried substance and dilute to 100.0 mL. Sonicate for 5 minutes.

Inject alternately 5 µL each of solutions (1), (2), (3) and (4).

Use the chromatograms obtained with solution (4) and solution (1) to identify the peak due to impurity A. Impurity A is eluted at the relative retention of about 0.8 with reference to methylthioninium (retention time about 11 minutes). The test is not valid unless the resolution between the peaks corresponding to methylthioninium and impurity A is at least 3.5.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity A is not greater than 5 times the area of the principal peak obtained with solution (2) (5.0%);
- the area of any other impurity peak is not greater than two times the area of the principal peak obtained with solution (3) (0.20%);
- the sum of the areas of all impurity peaks, other than the peak corresponding to impurity A, is not greater than the area of the principal peak obtained with solution (2) (1.0%). Disregard any peak with an area less than 0.5 times the area of the principal peak obtained with solution (3) (0.05%).

**Assay**

- Either method A or B may be applied.

**A.** Carry out test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (10 cm x 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded phenylsilyl groups (3.5 µm).¹

Use the following conditions for gradient elution:

- mobile phase A: 0.1% (v/v) solution of trifluoroacetic acid R;
- mobile phase B: acetonitrile R.

---

¹ An X-Bridge Phenyl column and a Phenomenex Luna 3 µm Phenyl-Hexyl column were found suitable.
<table>
<thead>
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</tr>
<tr>
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<td>Return to initial composition</td>
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<tr>
<td>35–40</td>
<td>80</td>
<td>20</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

Operate with a flow of 1.0 mL/min. As a detector us an ultraviolet spectrophotometer set at a wavelength of 246 nm. Maintain the column temperature at 30°C.

Prepare the following solutions using as diluent a mixture of 30 volumes acetonitrile R and 70 volumes of mobile phase A. For solution (1) dilute 5.0 mL of the injection to 50.0 mL. Dilute 5.0 mL of this solution to 50.0 mL to obtain a solution with a concentration of 0.1 mg of methylthioninium chloride per mL. For solution (2) dissolve 50.0 mg of methylthioninium chloride RS in 50.0 mL. Sonicate for 5 minutes. Dilute 5.0 mL of this solution to 50.0 mL.

Inject alternately 5 µL each of solutions (1) and (2). The test is not valid unless symmetry factor is not more than 2.0.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2) and calculate the percentage content of methylthioninium chloride \( \text{C}_{16}\text{H}_{18}\text{ClN}_{3}\text{S} \) using the declared content of \( \text{C}_{16}\text{H}_{18}\text{ClN}_{3}\text{S} \) in methylthioninium chloride RS.

B. Prepare the following solutions using as diluent ethanol (~457 g/L) TS. Dilute 1.0 mL of the injection to 100.0 mL to obtain a solution with a concentration of 0.1 mg of methylthioninium chloride per mL. Dilute 2.0 mL of this solution to 100.0 mL. Measure the absorbance \( \text{A} \) of a 1 cm layer of the diluted solution at the maximum at about 664 nm and calculate the percentage content of methylthioninium chloride \( \text{C}_{16}\text{H}_{18}\text{ClN}_{3}\text{S} \) using the absorptivity value of 2950 methylthioninium chloride.

**Note from the Secretariat.** The absorptivity value is so far based on a single determination. It is intended to perform further independent determinations to confirm the value.

**Bacterial endotoxins.** Carry out the test as described under 3.4 Test for bacterial endotoxins; contains less than 2.5 IU of endotoxin per mg methylthioninium chloride.

**Impurities**

The impurities limited by the requirements of this monograph include those listed in the monograph for methylthioninium chloride.

**Reagent to be established**

**Methylthioninium chloride R**

Methylthioninium chloride of a suitable quality should be used.
Proposed revision of the general chapter  

1.11 Colour of liquids

This is a proposed revision of the general chapter 1.11 Colour of liquids for The International Pharmacopoeia (Working document QAS/16.659, July 2016).

The working document with line numbers is available for comment at www.who.int/ pharmaceuticals/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.

[Note from the Secretariat.]

Addition of the test Degree of coloration of liquids (1.11.2), reproduced from the European Pharmacopoeia

In the current procedure for the preparation of the four colour stock standard test solutions used in chapter 1.11 Colour of liquid (yellow stock standard TS, red stock standard TS, green stock standard TS and brown stock standard TS) dichromate colour TS is used.

In order to replace chromium (VI) salts it is propose to gradually replace the existing procedure with the one used in the European Pharmacopoeia. (The permission to reproduce the procedure will be requested when the proposed text is adopted by the WHO Expert Committee on Specifications for Pharmaceutical Preparations.)

For the period of transition, the original text shall be kept under the section 1.11.1; the new test procedure shall be added under the section 1.11.2.

With the publication of the proposed revision of chapter 1.11 Colour of liquid, references to this chapter in existing monographs will be replaced with the reference to chapter 1.11.1. In new and revised monographs the new test procedure 1.11.2 will be applied.

The definition of “colourless” under General Notices

In the section General Notices a colourless solution is defined by referring to chapter 1.11 Colour of liquids as follows:

“A solution is considered colourless if it is not more intensely coloured than any of the standard colour solutions Bn0, Yw0, Gn0, or Rd0. The matching is made with the solution of most appropriate hue as described under 1.11 Colour of liquids.”

The specification colourless is mostly used in the sections “Clarity and colour of solution” of monographs, with and without explicit reference to chapter 1.11 Colour of liquids.

For the sake of clarity, it is proposed to delete the definition of “colourless” under General Notes and to put the definition of “colourless” under 1.11.1. Those specifications under “clarity and colour of solution” that currently do not refer to chapter 1.11 Colour of liquids are to be supplemented by a respective reference.]

[Note from the editor. In accordance with WHO editorial policy the text reproduced below does not include tracked changes. Changes from the current monograph are indicated by insert and delete in the working document available at the above-mentioned web address.]

1.11 Colour of liquid

In order to replace chromium (VI) salts in The International Pharmacopoeia the procedure previously used to determine the colour of liquids will be replaced gradually with the corresponding procedure taken over from the European Pharmacopoeia.

For the period of transition, both procedures are kept: the previous procedure under section 1.11.1 and the new procedure under section 1.11.2.
1.11.1 Colour of liquids

The test for colour of liquids is carried out by comparing the test solution prepared as specified in the monograph with a standard colour solution indicated in the monograph. The composition of the standard colour solution is selected depending on the hue and intensity of the colour of the test solution corresponding to the limits permitted in the specifications.

**Recommended procedure**

Unless otherwise specified in the monograph, carry out the comparison in flat-bottomed tubes of transparent glass that are matched as closely as possible in internal diameter and in all other respects (tubes of about 16 mm internal diameter are suitable). Use 10 mL of the test solution and 10 mL of the standard colour solution; the depth of liquid should be about 50 mm. The colour of the test solution is not more intense than the standard colour when viewed down the vertical axis of the tubes in diffused light against a white background.

**Stock colour standard solutions**

**Yellow stock standard TS**

To 9.5 mL of cobalt colour TS, add 1.9 mL of copper colour TS, 10.7 mL of dichromate colour TS, 4.0 mL of iron colour TS, dilute to 100.0 mL with sulfuric acid (~10 g/L) TS and mix.

**Red stock standard TS**

To 40.5 mL of cobalt colour TS, add 6.1 mL of copper colour TS, 6.3 mL of dichromate colour TS, 12.0 mL of iron colour TS, dilute to 100.0 mL with sulfuric acid (~10 g/L) TS and mix.

**Green stock standard TS**

To 3.5 mL of cobalt colour TS, add 20.1 mL of copper colour TS, 10.4 mL of dichromate colour TS, 4.0 mL of iron colour TS, dilute to 100.0 mL with sulfuric acid (~10 g/L) TS and mix.

**Brown stock standard TS**

To 35.0 mL of cobalt colour TS, add 17.0 mL of copper colour TS, 8.0 mL of dichromate colour TS, dilute to 100.0 mL with iron colour TS and mix.

**Standard colour solutions**

The standard colour solution is prepared by suitably diluting the stock standard solutions (yellow, red, green and brown stock standard TS) with sulfuric acid (~10 g/L) TS. The designation of the standard colour solution is composed of two letters indicating the stock standard solution (Yw for yellow, Rd for red, Gn for green and Bn for brown) and of a number describing the dilution as given below:

<table>
<thead>
<tr>
<th>Dilution number for standard colour solutions</th>
<th>Stock standard solution (mL)</th>
<th>Sulfuric acid (~10g/L) TS (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.78</td>
<td>99.22</td>
</tr>
<tr>
<td>1</td>
<td>1.56</td>
<td>98.44</td>
</tr>
<tr>
<td>2</td>
<td>3.12</td>
<td>96.88</td>
</tr>
<tr>
<td>3</td>
<td>6.25</td>
<td>93.75</td>
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<tr>
<td>4</td>
<td>12.50</td>
<td>87.50</td>
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<td>5</td>
<td>25.00</td>
<td>75.00</td>
</tr>
<tr>
<td>6</td>
<td>50.00</td>
<td>50.00</td>
</tr>
<tr>
<td>7</td>
<td>100.00</td>
<td>-</td>
</tr>
</tbody>
</table>

Standard colour solution numbers 4–7 may be stored in sealed glass containers, protected from sunlight but the more dilute standard colour solutions should be prepared as required.
Definition of “colourless”
A solution is considered colourless if it is not more intensely coloured than any of the standard colour solutions Bn0, Yw0, Gn0 or Rd0.

1.11.2 Degree of colouration of liquids

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The examination of the degree of coloration of liquids in the range brown-yellow-red is carried out by one of the 2 methods below as prescribed in the monograph.

A solution is colourless if it has the appearance of water R or the solvent or is not more intensely coloured than reference solution B9.

METHOD I
Using identical tubes of colourless, transparent, neutral glass of 12 mm external diameter, compare 2.0 mL of the liquid to be examined with 2.0 mL of water R or of the solvent or of the reference solution (see tables of reference solutions) prescribed in the monograph. Compare the colours in diffused daylight, viewing horizontally against a white background.

METHOD II
Using identical tubes of colourless, transparent, neutral glass with a flat base and an internal diameter of 15 mm to 25 mm, compare the liquid to be examined with water R or the solvent or the reference solution (see tables of reference solutions) prescribed in the monograph, the depth of the layer being 40 mm. Compare the colours in diffused daylight, viewing vertically against a white background.

REAGENTS

Primary solutions

Yellow solution. Dissolve 46 g of ferric chloride R in about 900 mL of a mixture of 25 mL of hydrochloric acid (~330 g/L) TS and 975 mL of water R and dilute to 1000.0 mL with the same mixture. Titrate and adjust the solution to contain 45.0 mg of FeCl₃·6H₂O per mL by adding the same acidic mixture. Protect the solution from light. Titration. Place in a 250 mL conical flask fitted with a ground-glass stopper, 10.0 mL of the solution, 15 mL of water R, 5 mL of hydrochloric acid (~330 g/L) TS and 4 g of potassium iodide R, close the flask, allow to stand in the dark for 15 minutes and add 100 mL of water R. Titrate the liberated iodine with sodium thiosulfate (0.1 mol/L) VS, using 0.5 mL of starch solution TS, added towards the end of the titration, as indicator. 1 mL of sodium thiosulfate (0.1 mol/L) VS is equivalent to 27.03 mg of FeCl₃·6H₂O.

Red solution. Dissolve 60 g of cobalt (II) chloride R in about 900 mL of a mixture of 25 mL of hydrochloric acid (~330 g/L) TS and 975 mL of water R and dilute to 1000.0 mL with the same mixture. Titrate and adjust the solution to contain 59.5 mg of CoCl₂·6H₂O per mL by adding the same acidic mixture.
Titration. Place in a 250 mL conical flask fitted with a ground-glass stopper, 5.0 mL of the solution, 5 mL of hydrogen peroxide (~30 g/L) TS and 10 mL of sodium hydroxide (~300 g/L) TS. Boil gently for 10 minutes, allow to cool and add 60 mL of sulfuric acid (~100 g/L) TS and 2 g of potassium iodide R. Close the flask and dissolve the precipitate by shaking gently. Titrate the liberated iodine with sodium thiosulfate (0.1 mol/L) VS, using 0.5 mL of starch solution TS, added towards the end of the titration, as indicator. The end-point is reached when the solution turns pink.

1 mL of sodium thiosulfate (0.1 mol/L) VS is equivalent to 23.79 mg of CoCl₂·6H₂O.

**Blue solution.** Dissolve 63 g of copper (II) sulfate R in about 900 mL of a mixture of 25 mL of hydrochloric acid (~330 g/L) TS and 975 mL of water R and dilute to 1000.0 mL with the same mixture. Titrate and adjust the solution to contain 62.4 mg of CuSO₄·5H₂O per mL by adding the same acidic mixture.

Titration. Place in a 250 mL conical flask fitted with a ground-glass stopper, 10.0 mL of the solution, 50 mL of water R, 12 mL of acetic acid (~120 g/L) TS and 3 g of potassium iodide R. Titrate the liberated iodine with sodium thiosulfate (0.1 mol/L) VS, using 0.5 mL of starch solution TS, added towards the end of the titration, as indicator. The end-point is reached when the solution shows a slight pale brown colour.

1 mL of sodium thiosulfate (0.1 mol/L) VS is equivalent to 24.97 mg of CuSO₄·5H₂O.

**Standard solutions**

Using the 3 primary solutions, prepare the 5 standard solutions as follows (Table 1).

<table>
<thead>
<tr>
<th>Standard solution</th>
<th>Volumes in mL</th>
<th>Hydrochloric acid (~10 g/L) TS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BY (brownish-yellow)</td>
<td>2.4</td>
<td>1.0</td>
</tr>
<tr>
<td>Y (yellow)</td>
<td>2.4</td>
<td>0.6</td>
</tr>
<tr>
<td>GY (greenish-yellow)</td>
<td>9.6</td>
<td>0.2</td>
</tr>
<tr>
<td>R (red)</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>B (brown)</td>
<td>3.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

**Reference solutions for Methods I and II**

Using the 5 standard solutions, prepare the following reference solutions.

<table>
<thead>
<tr>
<th>Reference solution</th>
<th>Volumes in mL</th>
<th>Hydrochloric acid (~10 g/L) TS</th>
</tr>
</thead>
<tbody>
<tr>
<td>B₁</td>
<td>75.0</td>
<td>25.0</td>
</tr>
<tr>
<td>B₂</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>B₃</td>
<td>37.5</td>
<td>62.5</td>
</tr>
<tr>
<td>B₄</td>
<td>25.0</td>
<td>75.0</td>
</tr>
<tr>
<td>B₅</td>
<td>12.5</td>
<td>87.5</td>
</tr>
<tr>
<td>B₆</td>
<td>5.0</td>
<td>95.0</td>
</tr>
<tr>
<td>B₇</td>
<td>2.5</td>
<td>97.5</td>
</tr>
<tr>
<td>B₈</td>
<td>1.5</td>
<td>98.5</td>
</tr>
<tr>
<td>B₉</td>
<td>1.0</td>
<td>99.0</td>
</tr>
</tbody>
</table>
### Table 3. Reference solutions BY

<table>
<thead>
<tr>
<th>Reference solution</th>
<th>Volumes in mL</th>
<th>Standard solution BY</th>
<th>Hydrochloric acid (~10 g/L) TS</th>
</tr>
</thead>
<tbody>
<tr>
<td>BY1</td>
<td>100.0</td>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td>BY2</td>
<td>75.0</td>
<td></td>
<td>25.0</td>
</tr>
<tr>
<td>BY3</td>
<td>50.0</td>
<td></td>
<td>50.0</td>
</tr>
<tr>
<td>BY4</td>
<td>25.0</td>
<td></td>
<td>75.0</td>
</tr>
<tr>
<td>BY5</td>
<td>12.5</td>
<td></td>
<td>87.5</td>
</tr>
<tr>
<td>BY6</td>
<td>5.0</td>
<td></td>
<td>95.0</td>
</tr>
<tr>
<td>BY7</td>
<td>2.5</td>
<td></td>
<td>97.5</td>
</tr>
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### Table 4. Reference solutions Y

<table>
<thead>
<tr>
<th>Reference solution</th>
<th>Volumes in mL</th>
<th>Standard solution Y</th>
<th>Hydrochloric acid (~10 g/L) TS</th>
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</thead>
<tbody>
<tr>
<td>Y1</td>
<td>100.0</td>
<td></td>
<td>0.0</td>
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<tr>
<td>Y2</td>
<td>75.0</td>
<td></td>
<td>25.0</td>
</tr>
<tr>
<td>Y3</td>
<td>50.0</td>
<td></td>
<td>50.0</td>
</tr>
<tr>
<td>Y4</td>
<td>25.0</td>
<td></td>
<td>75.0</td>
</tr>
<tr>
<td>Y5</td>
<td>12.5</td>
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<td>87.5</td>
</tr>
<tr>
<td>Y6</td>
<td>5.0</td>
<td></td>
<td>95.0</td>
</tr>
<tr>
<td>Y7</td>
<td>2.5</td>
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<td>97.5</td>
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### Table 5. Reference solutions GY

<table>
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<th>Volumes in mL</th>
<th>Standard solution GY</th>
<th>Hydrochloric acid (~10 g/L) TS</th>
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</thead>
<tbody>
<tr>
<td>GY1</td>
<td>25.0</td>
<td></td>
<td>75.0</td>
</tr>
<tr>
<td>GY2</td>
<td>15.0</td>
<td></td>
<td>85.0</td>
</tr>
<tr>
<td>GY3</td>
<td>8.5</td>
<td></td>
<td>91.5</td>
</tr>
<tr>
<td>GY4</td>
<td>5.0</td>
<td></td>
<td>95.0</td>
</tr>
<tr>
<td>GY5</td>
<td>3.0</td>
<td></td>
<td>97.0</td>
</tr>
<tr>
<td>GY6</td>
<td>1.5</td>
<td></td>
<td>98.5</td>
</tr>
<tr>
<td>GY7</td>
<td>0.75</td>
<td></td>
<td>99.25</td>
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### Table 6. Reference solutions R

<table>
<thead>
<tr>
<th>Reference solution</th>
<th>Volumes in mL</th>
<th>Standard solution R</th>
<th>Hydrochloric acid (~10 g/L) TS</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>100.0</td>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td>R2</td>
<td>75.0</td>
<td></td>
<td>25.0</td>
</tr>
<tr>
<td>R3</td>
<td>50.0</td>
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<td>R4</td>
<td>37.5</td>
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<td>62.5</td>
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<tr>
<td>R5</td>
<td>25.0</td>
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<td>75.0</td>
</tr>
<tr>
<td>R6</td>
<td>12.5</td>
<td></td>
<td>87.5</td>
</tr>
<tr>
<td>R7</td>
<td>5.0</td>
<td></td>
<td>95.0</td>
</tr>
</tbody>
</table>
Storage
For Method I the reference solutions may be stored in sealed tubes of colourless, transparent, neutral glass of 12 mm external diameter, protected from light.

For Method II prepare the reference solutions immediately before use from the standard solutions.

Reagents to be established

Starch solution TS
Triturate 1.0 g of soluble starch R with 5 mL of water R and whilst stirring pour the mixture into 100 mL of boiling water R containing 10 mg of mercuric iodide R.

NOTE: commercially available reagents may be used; including mercury-free solutions or those containing alternative preservatives.

Carry out the test for sensitivity each time the reagent is used.

Test for sensitivity. To a mixture of 1 mL of the starch solution and 20 mL of water R, add about 50 mg of potassium iodide R and 0.05 mL of iodine solution TS. The solution is blue. gents to be established

Hydrogen peroxide (~30 g/L) TS
A solution in water containing about 30 g of H2O2 per litre.

Hydrochloric acid (~10 g/L) TS
Hydrochloric acid (~250 g/L) TS, dilute with water to contain 10 g of HCl in 1000 mL.

Iodine solution TS
To 10.0 mL of M iodine (0.05 mol/L) VS add 0.6 g of potassium iodide R and dilute to 100.0 mL with water R. Prepare immediately before use.

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