CLINDAMYCIN PHOSPHATE
(CLINDAMYCINI PHOSPHAS)
REVISED DRAFT MONOGRAPH FOR INCLUSION IN
The International Pharmacopoeia
(August 2016)
DRAFT FOR COMMENTS

Should you have any comments on this draft, please send these to Dr Herbert Schmidt,
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Switzerland; fax: (+41 22) 791 4730 or email: schmidt@who.int by 21 October 2016.

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comments, please let us have your email address (to bonnyw@who.int) and we will add it
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**SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/16.678:**

Clindamycin phosphate (Clindamycini phosphas)

<table>
<thead>
<tr>
<th>Event</th>
<th>Date</th>
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<tbody>
<tr>
<td>Revision drafted by a WHO Collaborating Centre</td>
<td>October 2015–January 2016</td>
</tr>
<tr>
<td>Discussion at informal consultation on quality control laboratory tools and specifications for medicines</td>
<td>9–11 May 2016</td>
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<tr>
<td>Draft revision sent out for public consultation</td>
<td>August–October 2016</td>
</tr>
<tr>
<td>Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations for possible adoption</td>
<td>October 2016</td>
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<tr>
<td>Further follow-up action as required</td>
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</tbody>
</table>
CLINDAMYCIN PHOSPHATE

(CLINDAMYCINI PHOSPHAS)

[Note from the Secretariat. Changes from the current monograph are indicated in the text by insert or delete.]

\[
\text{C}_{18}\text{H}_{34}\text{ClN}_{2}\text{O}_{8}\text{PS}
\]

Relative molecular mass. 505.0

Chemical name

Methyl 7-Chloro-6,7,8-trideoxy-6-[[[(2S,4R)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]aminoo]-2-O-phosphono-1-thio-L-threo-α-D-galacto-octopyranoside (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-(dihydrogen phosphate); CAS Reg. No. 24729-96-2.

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 drug.

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

Labelling. The designation Clindamycin phosphate for parenteral use indicates that the substance complies with the additional requirements and may be used for parenteral administration. Expiry date.
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%–95.0% and not more than 102.0%–100.5% 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
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(-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; $\left[\alpha\right]_{D}^{20^\circ C} = +115^\circ$ to $+130^\circ$.

Clarity and colour of solution. A solution of 0.040 g in 10 mL of carbon dioxide free water R is clear and colourless. 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 0.5 g of the substance; the water content is not more than 0.050 0.060 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 below under "Assay". Inject alternately 20μl each of solutions A and D. Continue the recording of the chromatogram until clindamycin is eluted. Measure the areas of the peak responses obtained in the chromatograms from solutions A and D, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the principal peak or any peak corresponding to the solvent, is not greater than 2.5 times the area of the principal peak obtained with solution D (2.5%). The sum of the areas of all the peaks, other than the principal peak or any peak corresponding to the solvent, is not greater than 4 times the peak corresponding to clindamycin phosphate obtained with solution D (4.0%).

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:

¹ A Symmetry C18 column was found suitable.
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>
</tr>
<tr>
<td>13–18</td>
<td>100 to 50</td>
<td>0 to 50</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>18–39</td>
<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) 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. Determine 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 (5–10 μm). As the mobile phase, use a mixture of 8 volumes of potassium dihydrogen phosphate (13.6 g/l) TS adjusted to pH 2.5 with phosphoric acid (~105 g/l) TS and 2 volumes of acetonitrile R.

Prepare the following solutions in the mobile phase: solution (A) 3.0 mg of clindamycin phosphate per mL; solution (B) 3.0 mg of clindamycin phosphate RS per mL; for solution (C) dissolve 5 mg of lincomycin hydrochloride RS and 15.0 mg of clindamycin hydrochloride RS in 5.0 mL of solution B and dilute with sufficient mobile phase to produce 100 mL; and for solution (D) dilute 1.0 mL of solution B with sufficient mobile phase to produce 100 mL.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 210 nm.

Inject 20 μL of solution C.

The assay is not valid unless the first peak (lincomycin) is clearly separated from the solvent peak, and the resolution between the second peak (clindamycin phosphate) and the third peak (clindamycin) is at least 6.0. The assay is valid only if the symmetry factor of the clindamycin phosphate peak is not greater than 1.5.
Inject 20μl of solution B. If necessary adjust the integrator parameters.

Inject alternately 20μl each of solutions A and B.

Measure the areas of the peak responses obtained with solutions A and B, and calculate the percentage content of C₁₈H₃₄ClN₂O₈PS.

Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (15 cm x 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 (~450g/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 0.12mg 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 of the peaks corresponding to clindamycin phosphate obtained in the chromatograms from solutions (1) and (2) and calculate the percentage content of clindamycin phosphate (C₁₈H₃₄ClN₂O₈PS), using the declared content of clindamycin phosphate (C₁₈H₃₄ClN₂O₈PS) 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)

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]-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-propylidene pyrrolidin-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.