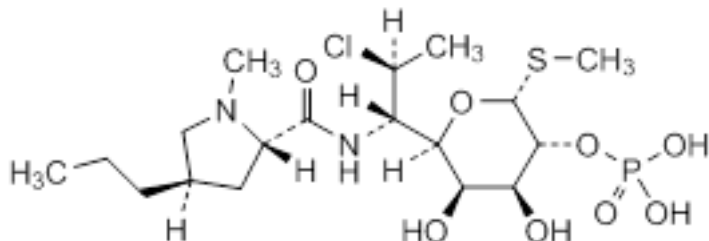


Clindamycin phosphate (Clindamycini phosphas)

2017-01

Molecular formula. C₁₈H₃₄ClN₂O₈PS**Relative molecular mass.** 505.0**Graphic formula****Chemical name.** methyl 7-chloro-6,7,8-trideoxy-6-[(2*S*,4*R*)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-β-*D*-galactopyranoside, 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.**Storage.** Clindamycin phosphate should be kept in a tightly closed container. If the substance is sterile, store in a sterile and air-tight container.**Additional information.** Clindamycin phosphate is slightly hygroscopic and may exhibit polymorphism. It is a semisynthetic product derived from a fermentation product.**Requirements****Definition.** Clindamycin phosphate contains not less than 96.0% and not more than 102.0% of C₁₈H₃₄ClN₂O₈PS, 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 phosphate RS or with the *reference spectrum* of clindamycin phosphate. If the spectra thus obtained are not concordant repeat the test. 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 Chromatography, 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 the 3 solutions in methanol R containing (A) 2.0 mg of Clindamycin phosphate per mL, (B) 2.0 mg of clindamycin phosphate RS per mL 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; $[\alpha]_D^{20} = +115^\circ$ to $+130^\circ$.

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.

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).

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 900 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.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0–13	100	0	Isocratic
13–18	100 to 50	0 to 50	Linear gradient
18–39	50	50	Isocratic
39–40	50 to 100	50 to 0	Return to initial composition
40–55	100	0	Re-equilibration

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 2 times the area of the principal peak in the chromatogram obtained with solution (3) (0.2%);
- 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 [1.14.1 Chromatography, 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]

A Symmetry C18 column was found suitable.

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 30.0 mg of the test substance and dilute to 10.0 mL. For solution (2) dissolve 30 mg of Clindamycin phosphate RS and dilute to 10.0 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 alternately 20 μL each of solutions (1) and (2).

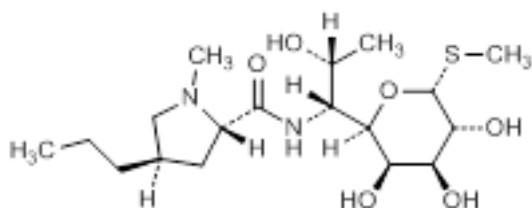
Measure the areas of the peaks corresponding to clindamycin phosphate obtained in the chromatograms from solutions (1) and (2) and calculate the percentage content of clindamycin phosphate (C₁₈H₃₄ClN₂O₈PS), using the declared content of clindamycin phosphate (C₁₈H₃₄ClN₂O₈PS) in clindamycin phosphate RS

Additional requirements for Clindamycin phosphate for parenteral use

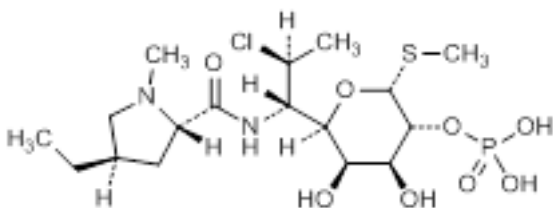
Complies with the monograph for [Parenteral preparations](#).

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.

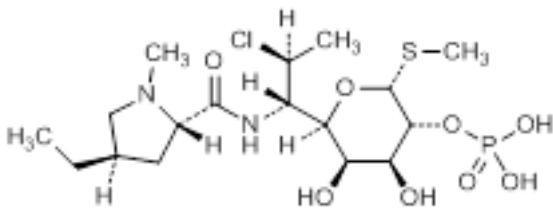
Impurities



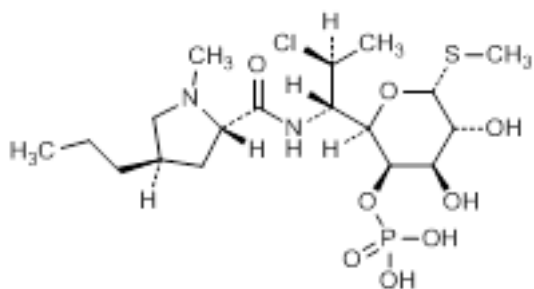
A. methyl 6,8-dideoxy-6-[(2S,4R)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-α-D-erythro-D-galacto-octopyranoside (lincomycin) (synthesis-related impurity, degradation product)



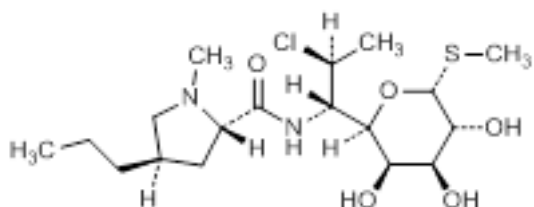
B. methyl 7-chloro-6,7,8-trideoxy-6-[(2S,4R)-4-ethyl-1-methylpyrrolidine-2-carboxamido]-1-thio-β-D-threo-D-galacto-octopyranoside, 2-(dihydrogen phosphate) (clindamycin B-2-phosphate) (synthesis-related impurity)



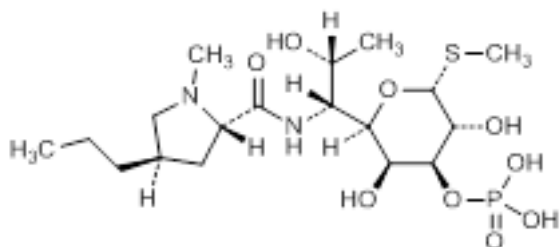
C. methyl 7-chloro-6,7,8-trideoxy-6-[(2S,4R)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-β-D-threo-D-galacto-octopyranoside, 3-(dihydrogen phosphate) (clindamycin-3-phosphate) (synthesis-related impurity)



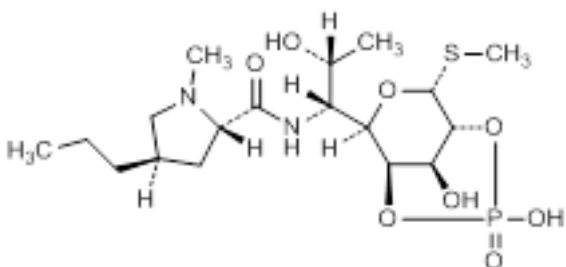
D. methyl 7-chloro-6,7,8-trideoxy-6-[(2*S*,4*R*)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-β-*l*-threo-*d*-galactooctopyranoside, 4-(dihydrogen phosphate) (clindamycin-4-phosphate) (synthesis-related impurity)



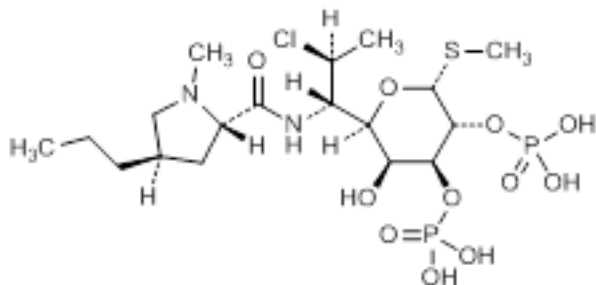
E. methyl 7-chloro-6,7,8-trideoxy-6-[(2*S*,4*R*)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-β-*l*-threo-*d*-galactooctopyranoside (clindamycin) (synthesis-related impurity/degradation product)



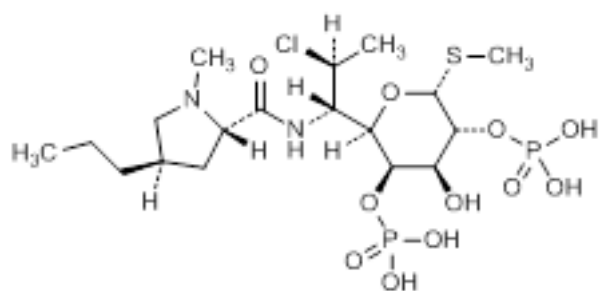
F. methyl 6,8-dideoxy-6-[(2*S*,4*R*)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-α-*d*-erythro-*d*-galactooctopyranoside, 2-(dihydrogen phosphate) (lincomycin 2-phosphate) (synthesis-related impurity, degradation product)



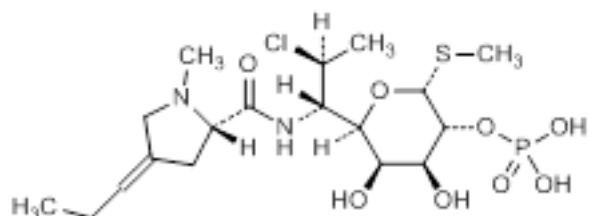
G. methyl 6,8-dideoxy-6-[(2*S*,4*R*)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-α-*d*-erythro-*d*-galactooctopyranoside, 2,4-(hydrogen phosphate) (lincomycin 2,4-phosphate) (synthesis-related impurity)



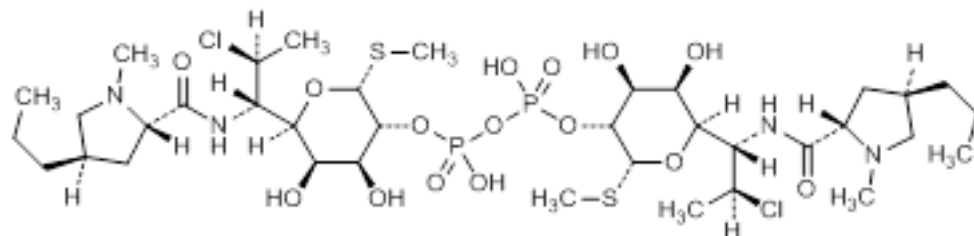
H. methyl 7-chloro-6,7,8-trideoxy-6-[(2*S*,4*R*)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-β-*l*-threo-*d*-galactooctopyranoside, 2,3-bis(dihydrogen phosphate) (clindamycin-2,3-bisphosphate) (synthesis-related impurity)



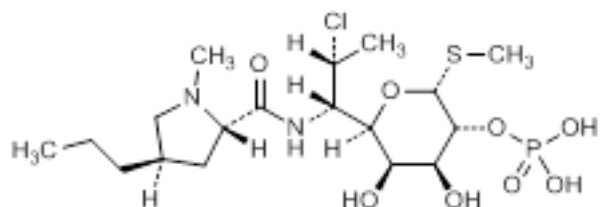
I. methyl 7-chloro-6,7,8-trideoxy-6-[(2S,4R)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-β-l-threo-d-galactooctopyranoside, 2,4-bis(dihydrogen phosphate) (clindamycin-2,4-bisphosphate)



J. methyl 7-chloro-6,7,8-trideoxy-6-[(2S,4R)-1-methyl-4-propyldenepyrrrolidine-2-carboxamido]-1-thio-β-l-threo-d-galactooctopyranoside, 2-(dihydrogen phosphate)



K. bis{methyl 7-chloro-6,7,8-trideoxy-6-[(2S,4R)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-β-l-threo-d-galactooctopyranoside}, 2,2'-(1,3-dihydrogen diphosphate)



L. methyl 7-chloro-6,7,8-trideoxy-6-[(2S,4R)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-α-d-erythro-d-galactooctopyranoside, 2-(dihydrogen phosphate) (7-*epi*-clindamycin phosphate) (synthesis-related impurity, degradation product)