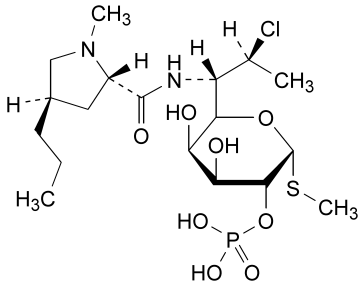
**01/2008:0996**
**corrected 6.0**

# CLINDAMYCIN PHOSPHATE

## Clindamycini phosphas



$C_{18}H_{34}ClN_2O_8PS$ $M_r$ 505.0
[24729-96-2]

DEFINITION

Methyl 7-chloro-6,7,8-trideoxy-6-[[[(2*S*,4*R*)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-L-*threo*-α-D-*galacto*-octopyranoside 2-(dihydrogen phosphate).

Semi-synthetic product derived from a fermentation product.

*Content*: 95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

*Appearance*: white or almost white, slightly hygroscopic powder.

*Solubility*: freely soluble in water, very slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

*First identification: A, D.*

*Second identification: B, C, D.*

A. Infrared absorption spectrophotometry (*2.2.24*).

*Preparation*: discs of *potassium bromide R*.

In 2 separate tubes place 50 mg of the substance to be examined and 50 mg of *clindamycin phosphate CRS*. Add 0.2 ml of *water R* and heat until completely dissolved. Evaporate to dryness under reduced pressure and dry the residues at 100-105 °C for 2 h.

*Comparison*: *clindamycin phosphate CRS*.

B. Thin-layer chromatography (*2.2.27*).

*Test solution*. Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

*Reference solution (a)*. Dissolve 20 mg of *clindamycin phosphate CRS* in *methanol R* and dilute to 10 ml with the same solvent.

*Reference solution (b)*. Dissolve 10 mg of *lincomycin hydrochloride CRS* in 5 ml of reference solution (a).

*Plate*: *TLC silica gel plate R*.

*Mobile phase*: *glacial acetic acid R, water R, butanol R* (20:20:60 *V/V/V*).

*Application*: 5 µl.

*Development*: over a path of 12 cm.

*Drying*: at 100-105 °C for 30 min.

*Detection*: spray with a 1 g/l solution of *potassium permanganate R*.

*System suitability*: reference solution (b):

– the chromatogram shows 2 principal spots.

*Results*: the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve about 10 mg in 2 ml of *dilute hydrochloric acid R* and heat in a water-bath for 3 min. Add 4 ml of *sodium carbonate solution R* and 1 ml of a 20 g/l solution of *sodium nitroprusside R*. Prepare a standard in the same manner using *clindamycin phosphate CRS*. The colour of the test solution corresponds to that of the standard.

D. Boil 0.1 g under a reflux condenser with a mixture of 5 ml of *strong sodium hydroxide solution R* and 5 ml of *water R* for 90 min. Cool and add 5 ml of *nitric acid R*. Extract with 3 quantities, each of 15 ml, of *methylene chloride R* and discard the extracts. Filter the upper layer through a paper filter. The filtrate gives reaction (b) of phosphates (*2.3.1*).

TESTS

**Solution S**. 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*.

**Appearance of the solution**. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

**pH** (*2.2.3*): 3.5 to 4.5.

Dilute 5.0 ml of solution S to 20 ml with *carbon dioxide-free water R*.

**Specific optical rotation** (*2.2.7*): + 115 to + 130 (anhydrous substance).

Dissolve 0.250 g in *water R* and dilute to 25.0 ml with the same solvent.

**Related substances**. Liquid chromatography (*2.2.29*).

*Test solution*. Dissolve 75.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

*Reference solution (a)*. Dissolve 75.0 mg of *clindamycin phosphate CRS* in the mobile phase and dilute to 25.0 ml with the mobile phase.

*Reference solution (b)*. Dissolve 5.0 mg of *lincomycin hydrochloride CRS* (impurity A) and 15.0 mg of *clindamycin hydrochloride CRS* (impurity E) in 5.0 ml of reference solution (a), then dilute to 100.0 ml with the mobile phase.

*Reference solution (c)*. Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

*Column*:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;

– *stationary phase*: *octylsilyl silica gel for chromatography R* (5-10 µm).

*Mobile phase*: mix 200 ml of *acetonitrile R1* and 800 ml of a 13.6 g/l solution of *potassium dihydrogen phosphate R* previously adjusted to pH 2.5 with *phosphoric acid R*.

*Flow rate*: 1.0 ml/min.

*Detection*: spectrophotometer at 210 nm.

*Injection*: 20 µl of the test solution and reference solutions (b) and (c).

*Run time*: the retention time of impurity E.

*System suitability*: reference solution (b):

– *resolution*: minimum 6.0 between the peaks due to clindamycin phosphate (2nd peak) and impurity E (3rd peak); if necessary, adjust the concentration of acetonitrile in the mobile phase;

– *symmetry factor*: maximum 1.5 for the peak due to clindamycin phosphate;
– the peak due to impurity A (1st peak) is clearly separated from the peak due to the solvent.

*Limits*:

– *any impurity*: for each impurity, not more than 2.5 times the area of the peak due to clindamycin phosphate in the chromatogram obtained with reference solution (c) (2.5 per cent);
– *total*: not more than 4 times the area of the peak due to clindamycin phosphate in the chromatogram obtained with reference solution (c) (4.0 per cent);
– *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

**Water** (*2.5.12*): maximum 6.0 per cent, determined on 0.250 g.

**Bacterial endotoxins** (*2.6.14*): less than 0.6 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

*Injection*: the test solution and reference solution (a).

*System suitability*: reference solution (a):

– *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections; if necessary, adjust the integrator parameters.

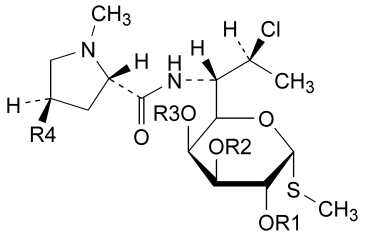
Calculate the percentage content of $C_{18}H_{34}ClN_2O_8PS$ from the declared content of *clindamycin phosphate CRS*.

STORAGE

In an airtight container, at a temperature not exceeding 30 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES

A. lincomycin,




B. R1 = $PO_3H_2$, R2 = R3 = H, R4 = $C_2H_5$: clindamycin B 2-(dihydrogen phosphate),

C. R1 = R3 = H, R2 = $PO_3H_2$, R4 = $C_3H_7$: clindamycin 3-(dihydrogen phosphate),

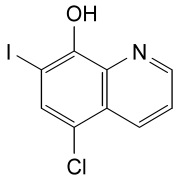
D. R1 = R2 = H, R3 = $PO_3H_2$, R4 = $C_3H_7$: clindamycin 4-(dihydrogen phosphate),

E. R1 = R2 = R3 = H, R4 = $C_3H_7$: clindamycin.

**01/2008:2111**

# CLIOQUINOL

## Clioquinolum




$C_9H_5ClINO$ $M_r$ 305.5
[130-26-7]

DEFINITION

5-Chloro-7-iodoquinolin-8-ol.

*Content*: 98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

*Appearance*: almost white, light yellow, brownish-yellow or yellowish-grey powder.

*Solubility*: practically insoluble in water, sparingly soluble in methylene chloride, very slightly soluble or slightly soluble in ethanol (96 per cent).

IDENTIFICATION

*First identification: B.*

*Second identification: A, C, D.*

A. Dissolve 40.0 mg in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml to 100.0 ml with *methanol R* (solution A). Examined between 280 nm and 350 nm (*2.2.25*), solution A shows an absorption maximum at 321 nm. Dilute 10.0 ml of solution A to 100.0 ml with *methanol R* (solution B). Examined between 230 nm and 280 nm, solution B shows an absorption maximum at 255 nm. The specific absorbance at this absorption maximum is 1530 to 1660.

B. Infrared absorption spectrophotometry (*2.2.24*).
*Preparation*: discs of *potassium bromide R*.
*Comparison*: *clioquinol CRS*.

C. When heated, violet fumes are produced.

D. Dissolve about 1 mg in 5 ml of *ethanol (96 per cent) R*. Add 0.05 ml of *ferric chloride solution R1*. A dark green colour develops.

TESTS

**Acidity or alkalinity**. Shake 0.5 g with 10 ml of *carbon dioxide-free water R* and filter. To the filtrate add 0.2 ml of *phenolphthalein solution R*. The solution is colourless. Not more than 0.5 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink.

**Related substances**. Liquid chromatography (*2.2.29*).

*Test solution*. Dissolve 50.0 mg of the substance to be examined in *methanol R* and dilute to 50.0 ml with the same solvent, heating gently if necessary. Dilute 10.0 ml of the solution to 25.0 ml with the mobile phase.

*Reference solution (a)*. Dissolve 20.0 mg of *5-chloroquinolin-8-ol R*, 10.0 mg of *5,7-dichloroquinolin-8-ol R*, 5 mg of the substance to be examined and 10.0 mg of *5,7-diiodoquinolin-8-ol R* in *methanol R*, heating gently if necessary and dilute to 20.0 ml with the same solvent. Dilute 4.0 ml of the solution to 50.0 ml with the mobile phase.

*Reference solution (b)*. Dilute 1.0 ml of reference solution (a) to 10.0 ml with the mobile phase.