Appearance of solution. Solution S is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

pH (*2.2.3*): 5.0 to 7.0 for solution S.

Optical rotation (*2.2.7*): – 0.10° to + 0.10°.

Dissolve 0.30 g in *water R* and dilute to 10.0 ml with the same solvent. Filter if necessary.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Disperse 100.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a). Dilute 0.1 ml of the test solution to 100.0 ml with *water R*.

Reference solution (b). Dissolve 5 mg of *clenbuterol impurity B CRS* in 10 ml of the mobile phase, add 2.5 ml of the test solution and dilute to 25.0 ml with the mobile phase.

Column:

– *size*: *l* = 0.125 m, Ø = 4 mm,
– *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm),
– *temperature*: 40 °C.

Mobile phase: mix 200 volumes of *acetonitrile R*, 200 volumes of *methanol R* and 600 volumes of a solution prepared as follows: dissolve 3.0 g of *sodium decanesulphonate R* and 5.0 g of *potassium dihydrogen phosphate R* in 900 ml of *water R*, adjust to pH 3.0 with *dilute phosphoric acid R* and dilute to 1000 ml with *water R*.

Flow rate: 0.5 ml/min.

Detection: spectrophotometer at 215 nm.

Injection: 5 µl.

Run time: 1.5 times the retention time of clenbuterol .

Retention time: clenbuterol = about 29 min.

System suitability: reference solution (b):

– *resolution*: minimum 4.0 between the peaks due to impurity B and clenbuterol.

Limits:

– *impurities A, B, C, D, E, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
– *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
– *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent),
– *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*): maximum 1.0 per cent, determined on 0.500 g.

Sulphated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

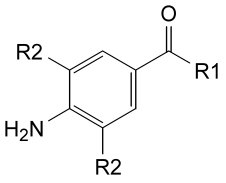
ASSAY

Dissolve 0.250 g in 50 ml of *ethanol (96 per cent) R* and add 5.0 ml of *0.01 M hydrochloric acid*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 31.37 mg of $C_{12}H_{19}Cl_3N_2O$.

IMPURITIES

Specified impurities: A, B, C, D, E, F.



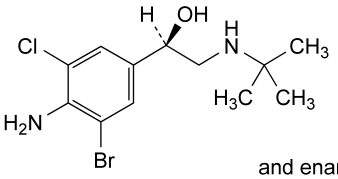
A. R1 = H, R2 = Cl: 4-amino-3,5-dichlorobenzaldehyde,

B. R1 = CH_2-NH-$C(CH_3)_3$, R2 = Cl: 1-(4-amino-3,5-dichlorophenyl)-2-[(1,1-dimethylethyl)amino]ethanone,

C. R1 = CH_3, R2 = Cl: 1-(4-amino-3,5-dichlorophenyl)ethanone,

D. R1 = CH_3, R2 = H: 1-(4-aminophenyl)ethanone,

E. R1 = CH_2Br, R2 = Cl: 1-(4-amino-3,5-dichlorophenyl)-2-bromoethanone,



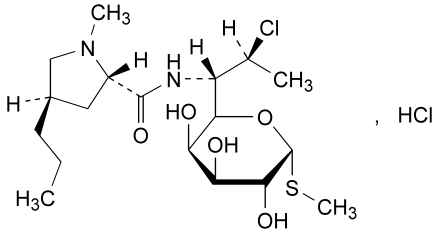
and enantiomer

F. (1*RS*)-1-(4-amino-3-bromo-5-chlorophenyl)-2-[(1,1-dimethylethyl)amino]ethanol.

01/2008:0582
corrected 6.0

CLINDAMYCIN HYDROCHLORIDE

Clindamycini hydrochloridum



, HCl

$C_{18}H_{34}Cl_2N_2O_5S$ M_r 461.5
[21462-39-5]

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 hydrochloride. It contains a variable quantity of water.

Semi-synthetic product derived from a fermentation product.

Content: 91.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: very soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: A, D.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *clindamycin hydrochloride CRS*.

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

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

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

Reference solution (b). Dissolve 10 mg of *clindamycin hydrochloride CRS* and 10 mg of *lincomycin hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate: *TLC silica gel G plate R.*

Mobile phase: mix 19 volumes of *2-propanol R*, 38 volumes of a 150 g/l solution of *ammonium acetate R* adjusted to pH 9.6 with *ammonia R*, and 43 volumes of *ethyl acetate R.*

Application: 5 µl.

Development: over a path of 15 cm using the upper layer of the mobile phase.

Drying: in air.

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

System suitability: the chromatogram obtained with reference solution (b) shows 2 clearly separated 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 on a water-bath for 3 min. Add 3 ml of *sodium carbonate solution R* and 1 ml of a 20 g/l solution of *sodium nitroprusside R.* A violet-red colour develops.

D. Dissolve 0.1 g in *water R* and dilute to 10 ml with the same solvent. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

pH (*2.2.3*): 3.0 to 5.0.

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Specific optical rotation (*2.2.7*): + 135 to + 150 (anhydrous substance).

Dissolve 1.000 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a). Dissolve 50.0 mg of *clindamycin hydrochloride CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (b). Dilute 2.0 ml of the test solution to 100.0 ml with the mobile phase.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm,
– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: mix 45 volumes of *acetonitrile R* and 55 volumes of a 6.8 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 7.5 with a 250 g/l solution of *potassium hydroxide R.*

Flow rate: 1 ml/min.

Detection: spectrophotometer at 210 nm.

Injection: 20 µl.

Run time: twice the retention time of clindamycin.

System suitability: reference solution (a):

– *relative retention* with reference to clindamycin (retention time = about 10 min): impurity A = about 0.4; impurity B = about 0.65; impurity C = about 0.8.

Limits:

– *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent),
– *impurity C*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (4.0 per cent),
– *any other impurity*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent),
– *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (6.0 per cent),
– *disregard limit*: 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.12*): 3.0 per cent to 6.0 per cent, determined on 0.500 g.

Sulphated ash (*2.4.14*): maximum 0.5 per cent, determined on 1.0 g.

ASSAY

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

Injection: 20 µl of the test solution and reference solution (a).

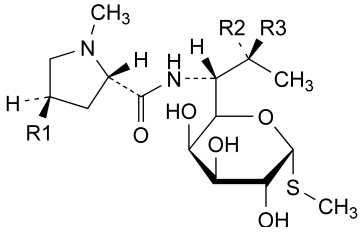
System suitability:

– *repeatability*: maximum relative standard deviation of 0.85 per cent after 6 injections of reference solution (a).

STORAGE

In an airtight container.

IMPURITIES



A. R1 = CH_2-CH_2-CH_3, R2 = OH, R3 = H: methyl 6,8-dideoxy-6-[[[(2*S*,4*R*)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside (lincomycin),

B. R1 = C_2H_5, R2 = H, R3 = Cl: methyl 7-chloro-6,7,8-trideoxy-6-[[[(2*S*,4*R*)-4-ethyl-1-methylpyrrolidin-2-yl]carbonyl]amino]-1-thio-L-*threo*-α-D-*galacto*-octopyranoside (clindamycin B),

C. R1 = CH_2-CH_2-CH_3, R2 = Cl, R3 = H: methyl 7-chloro-6,7,8-trideoxy-6-[[[(2*S*,4*R*)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside (7-epiclindamycin).