a brownish-yellow fluorescent zone. Below this zone, 2 yellow fluorescent zones may be present. Between the zones due to rutin and hyperoside, orange and yellow fluorescent zones are visible. Between the zones due to hyperoside and caffeic acid, up to 5 yellow or orange fluorescent zones are present. Immediately below the zone due to caffeic acid is a a blue fluorescent zone.

TESTS

Foreign matter (*2.8.2*): maximum 2 per cent, determined on 30 g. There are no inflorescences with a bract bearing at the abaxial face stellate, five- to eight-rayed trichomes and flowers having an apparent double corolla by transformation of five stamens into petal-like staminoids and having a pistil which is not lobular nor indented. Hexamerous flowers occur only occasionally (*Tilia americana* L., *Tilia tomentosa* Moench).

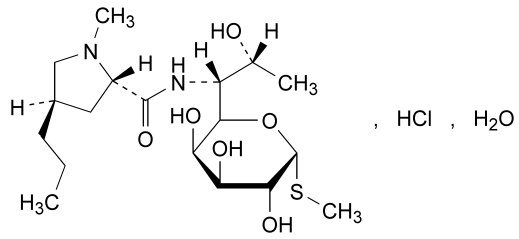
Loss on drying (*2.2.32*): maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*): maximum 8.0 per cent.

01/2008:0583

LINCOMYCIN HYDROCHLORIDE

Lincomycini hydrochloridum



, HCl , H_2O

$C_{18}H_{35}ClN_2O_6S,H_2O$ M_r 461.0
[7179-49-9]

DEFINITION

Lincomycin hydrochloride consists mainly of the 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 hydrochloride, an antimicrobial substance produced by *Streptomyces lincolnensis* var. *lincolnensis* or by any other means. It contains not less than 89.5 per cent and not more than 102.0 per cent of lincomycin hydrochloride ($C_{18}H_{35}ClN_2O_6S$), calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white, crystalline powder, very soluble in water, slightly soluble in ethanol (96 per cent), very slightly soluble in acetone.

IDENTIFICATION

First identification: A, D.

Second identification: B, C, D.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *lincomycin hydrochloride CRS*.

B. Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

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 *lincomycin hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

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

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using the upper layer from a mixture of 20 volumes of *2-propanol R*, 40 volumes of a 150 g/l solution of *ammonium acetate R* previously adjusted to pH 9.6 with *ammonia R* and 45 volumes of *ethyl acetate R*. Allow the plate to dry in air and spray with a 1 g/l solution of *potassium permanganate R*. 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). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. Dissolve about 10 mg in 2 ml of *dilute hydrochloric acid R* and heat in 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

Solution S. Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

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

pH (*2.2.3*). The pH of solution S is 3.5 to 5.5.

Specific optical rotation (*2.2.7*). Dissolve 1.000 g in *water R* and dilute to 25.0 ml with the same solvent. The specific optical rotation is + 135 to + 150, calculated with reference to the anhydrous substance.

Lincomycin B. Examine the chromatogram obtained in the assay with test solution (a). The area of the peak due to lincomycin B, which is eluted just before lincomycin, is not more than 5 per cent of the area of the peak due to lincomycin.

Heavy metals (*2.4.8*). 2.0 g complies with test C for heavy metals (5 ppm). Prepare the reference solution using 1.0 ml of *lead standard solution (10 ppm Pb) R*.

Water (*2.5.12*). 3.1 per cent to 4.6 per cent, determined on 0.500 g by the semi-micro determination of water.

Sulphated ash (*2.4.14*). Not more than 0.5 per cent, determined on 1.0 g.

Bacterial endotoxins (*2.6.14*): less than 0.50 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for removal of bacterial endotoxins.

ASSAY

Examine by gas chromatography (*2.2.28*), using *dotriacontane R* as the internal standard.

Internal standard solution. Dissolve 0.200 g of *dotriacontane R* in *chloroform R* and dilute to 25.0 ml with the same solvent.

Test solution (a). Dissolve 0.100 g of the substance to be examined in a 20 g/l solution of *imidazole R* in *chloroform R* and dilute to 100.0 ml with the same solution. Shake until

dissolution is complete. Place 4.0 ml of the solution in a ground-glass-stoppered 15 ml centrifuge tube. Add 1.0 ml of a mixture of 1 volume of *chlorotrimethylsilane R* and 99 volumes of *N,O-bis(trimethylsilyl)acetamide R* and swirl gently. Position the glass stopper loosely in the tube and heat at 65 °C for 30 min.

Test solution (b). Prepare as described for test solution (a) but add 10.0 ml of the internal standard solution before dissolution of the substance to be examined.

Reference solution. Prepare as described for test solution (a) using 0.100 g of *lincomycin hydrochloride CRS* instead of the substance to be examined and adding 10.0 ml of the internal standard solution before dissolution of the reference substance.

The chromatographic procedure may be carried out using:

– a glass column 1.5 m long and 3 mm in internal diameter packed with *silanised diatomaceous earth for gas chromatography R* impregnated with 3 per cent *m/m* of *poly(methylphenylsiloxane) R*,
– *helium for chromatography R* as the carrier gas at a flow rate of about 45 ml/min,
– a flame-ionisation detector,

maintaining the temperature of the column at 260 °C and that of the injection port and of the detector between 260 °C and 290 °C. Inject the chosen volume of the test solutions and the reference solution.

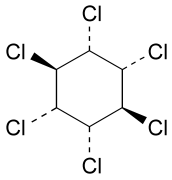
STORAGE

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

01/2008:0772

LINDANE

Lindanum



$C_6H_6Cl_6$ M_r 290.8
[58-89-9]

DEFINITION

Lindane contains not less than 99.0 per cent and not more than the equivalent of 100.5 per cent of *r*-1,*c*-2,*t*-3,*c*-4,*c*-5,*t*-6-hexachlorocyclohexane.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, freely soluble in acetone, soluble in ethanol.

IDENTIFICATION

First identification: A, B.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 112 °C to 115 °C.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *lindane CRS*. Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 5 mg in 4 ml of *alcohol R*. Add 1 ml of *0.5 M potassium hydroxide alcoholic*. Allow to stand for 10 min. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Appearance of solution. Dissolve 0.50 g in *acetone R* and dilute to 10 ml with the same solvent. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution B_7 (*2.2.2, Method II*).

Related substances. Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution (a). Dissolve 1.0 g of the substance to be examined in *chloroform R* and dilute to 10 ml with the same solvent.

Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with *chloroform R*.

Reference solution (a). Dissolve 0.1 g of *lindane CRS* in *chloroform R* and dilute to 10 ml with the same solvent.

Reference solution (b). Dilute 1 ml of test solution (b) to 10 ml with *chloroform R*.

Reference solution (c). Dissolve 10 mg of *α-hexachlorocyclohexane CRS* in test solution (a) and dilute to 5 ml with the same solvent.

Apply separately to the plate 1 µl of each solution. Develop over a path of 12 cm using a mixture of 10 volumes of *chloroform R* and 90 volumes of *cyclohexane R*. Dry the plate in a current of air, irradiate with ultraviolet light at 254 nm for 15 min and spray with a 6 g/l solution of *dicarboxidine hydrochloride R* in *alcohol (90 per cent V/V) R*. Examine in daylight. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.0 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Chlorides (*2.4.4*). To 0.75 g, finely powdered, add 15 ml of *water R*. Boil for 1 min. Allow to cool, shaking frequently, and filter. To 10 ml of the filtrate add 3 ml of *water R* and 2 ml of *alcohol R*. The solution complies with the limit test for chlorides (100 ppm).

Sulphated ash (*2.4.14*). Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

To 0.200 g add 10 ml of *alcohol R* and warm on a water-bath until dissolved. Cool, add 20 ml of *0.5 M alcoholic potassium hydroxide* and allow to stand for 10 min, swirling frequently. Add 50 ml of *water R*, 20 ml of *dilute nitric acid R*, 25.0 ml of *0.1 M silver nitrate* and 5 ml of *ferric ammonium sulphate solution R2*. Titrate with *0.1 M ammonium thiocyanate* until a reddish-yellow colour is obtained. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 9.694 mg of $C_6H_6Cl_6$.

STORAGE

Store protected from light.