– *impurities E, F*: for each impurity, not more than 0.1 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (f) (0.5 per cent),
– *any other impurity*: for each impurity, not more than 0.04 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (f) (0.2 per cent),
– *total*: not more than 1.6 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (f) (8.0 per cent),
– *disregard limit*: 0.02 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (f) (0.1 per cent).

Methanol (*2.4.24, System A*): maximum 1.5 per cent.

Water (*2.5.12*): maximum 5.0 per cent, determined on 0.200 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: test solution and reference solution (a).

System suitability:

– *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections of reference solution (a).

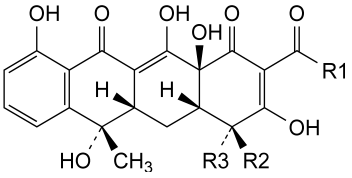
Calculate the percentage content of tetracycline and multiply it by 1.3560 to obtain the percentage content of lymecycline.

STORAGE

In an airtight container, protected from light.

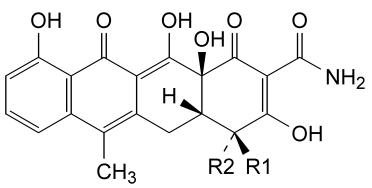
IMPURITIES

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



A. R1 = NH_2, R2 = H, R3 = $N(CH_3)_2$: (4*R*,4a*S*,5a*S*,6*S*,12a*S*)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epitetracycline),

B. R1 = CH_3, R2 = $N(CH_3)_2$, R3 = H: (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-2-acetyl-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-4a,5a,6,12a-tetrahydrotetracene-1,11(4*H*,5*H*)-dione (2-acetyl-2-decarbamoyltetracycline),



C. R1 = $N(CH_3)_2$, R2 = H: (4*S*,4a*S*,12a*S*)-4-(dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (anhydrotetracycline),

D. R1 = H, R2 = $N(CH_3)_2$: (4*R*,4a*S*,12a*S*)-4-(dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (4-epianhydrotetracycline),

E. unknown structure,

F. unknown structure,

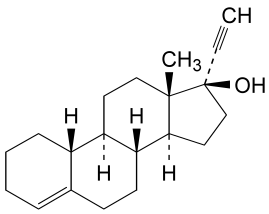
G. chlortetracycline,

H. tetracycline.

01/2008:0558
corrected 6.0

LYNESTRENOL

Lynestrenolum



$C_{20}H_{28}O$ M_r 284.4
[52-76-6]

DEFINITION

Lynestrenol contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of 19-nor-17α-pregn-4-en-20-yn-17-ol, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, soluble in acetone and in alcohol.

IDENTIFICATION

First identification: B.

Second identification: A, C.

A. Melting point (*2.2.14*): 161 °C to 165 °C.
B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *lynestrenol CRS*.
C. Examine the chromatograms obtained in the test for related substances in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with test solution (b) is similar in position, fluorescence and size to the principal spot in the chromatogram obtained with reference solution (b).

TESTS

Appearance of solution. Dissolve 0.2 g in *alcohol R* and dilute to 10 ml with the same solvent. The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*). Dissolve 0.900 g in *alcohol R* and dilute to 25.0 ml with the same solvent. The specific optical rotation is – 9.5 to – 11, calculated with reference to the dried substance.

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

Test solution (a). Dissolve 0.125 g of the substance to be examined in *chloroform R* and dilute to 25 ml with the same solvent.

Test solution (b). Dilute 5 ml of test solution (a) to 10 ml with *chloroform R*.

Reference solution (a). Dilute 1 ml of test solution (a) to 100 ml with *chloroform R*. Dilute 5 ml of the solution to 10 ml with *chloroform R*.

Reference solution (b). Dissolve 25 mg of *lynestrenol CRS* in *chloroform R* and dilute to 10 ml with the same solvent.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 20 volumes of *acetone R* and 80 volumes of *heptane R*. Allow the plate to dry in air, spray with *0.25 M alcoholic sulphuric acid R* and heat at 105 °C for 10 min. Examine in ultraviolet light at 365 nm. 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 (a) (0.5 per cent).

Loss on drying (*2.2.32*). Not more than 0.5 per cent, determined on 0.500 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.150 g in 40 ml of *tetrahydrofuran R* and add 5.0 ml of a 100 g/l solution of *silver nitrate R*. Titrate with *0.1 M sodium hydroxide*. Determine the end-point potentiometrically (*2.2.20*), using a glass indicator electrode and as comparison electrode a silver-silver chloride double-junction electrode with a saturated solution of *potassium nitrate R* as junction liquid. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 28.44 mg of $C_{20}H_{28}O$.

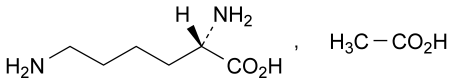
STORAGE

Store protected from light.

01/2008:2114

LYSINE ACETATE

Lysini acetas



$C_8H_{18}N_2O_4$ M_r 206.2
[57282-49-2]

DEFINITION

(2*S*)-2,6-Diaminohexanoic acid acetate.

Content: 98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder or colourless crystals.

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

IDENTIFICATION

First identification: A, B, E.

Second identification: A, C, D, E.

A. It complies with the test for specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *lysine acetate CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *water R*, evaporate to dryness at 60 °C and record new spectra using the residues.

C. Examine the chromatograms obtained in the test for ninhydrin-positive substances.

Results: 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. To 0.1 ml of solution S (see Tests) add 2 ml of *water R* and 1 ml of a 50 g/l solution of *phosphomolybdic acid R*. A yellowish-white precipitate is formed.

E. It gives reaction (a) of acetates (*2.3.1*).

TESTS

Solution S. Dissolve 5.0 g in *distilled water R* and dilute to 50 ml with the same solvent.

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

Specific optical rotation (*2.2.7*): + 8.5 to + 10.0 (dried substance), determined on solution S.

Ninhydrin-positive substances. Thin-layer chromatography (*2.2.27*).

Test solution (a). Dissolve 0.10 g of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Test solution (b). Dilute 1.0 ml of test solution (a) to 50 ml with *water R*.

Reference solution (a). Dissolve 10 mg of *lysine acetate CRS* in *water R* and dilute to 50 ml with the same solvent.

Reference solution (b). Dilute 5 ml of test solution (b) to 20 ml with *water R*.

Reference solution (c). Dissolve 10 mg of *lysine acetate CRS* and 10 mg of *arginine CRS* in *water R* and dilute to 25 ml with the same solvent.

Plate: *TLC silica gel plate R*.

Mobile phase: *concentrated ammonia R, 2-propanol R* (30:70 *V/V*).

Application: 5 µl.

Development: over 2/3 of the plate.

Drying: at 100-105 °C until the ammonia has evaporated.

Detection: spray with *ninhydrin solution R* and heat at 100-105 °C for 15 min.

System suitability: reference solution (c):

– the chromatogram shows 2 clearly separated spots.

Limits: test solution (a):

– *any impurity*: any spot, apart from the principal spot, is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Chlorides (*2.4.4*): maximum 200 ppm.

Dilute 2.5 ml of solution S to 15 ml with *water R*.

Sulphates (*2.4.13*): maximum 300 ppm.

Dilute 5 ml of solution S to 15 ml with *distilled water R*.

Ammonium (*2.4.1, Method B*): maximum 200 ppm, determined on 50 mg.

Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm NH_4) R*.