01/2008:0548
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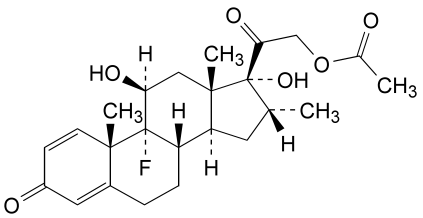
DEXAMETHASONE ACETATE

Dexamethasoni acetas



$C_{24}H_{31}FO_6$ M_r 434.5
[1177-87-3]

DEFINITION

9-Fluoro-11β,17-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl acetate.

Content: 97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

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

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification: B, C.

Second identification: A, C, D, E, F.

A. Dissolve 10.0 mg in *anhydrous ethanol R* and dilute to 100.0 ml with the same solvent. Place 2.0 ml of this solution in a ground-glass-stoppered tube, add 10.0 ml of *phenylhydrazine-sulphuric acid solution R*, mix and heat in a water-bath at 60 °C for 20 min. Cool immediately. The absorbance (*2.2.25*) measured at the absorption maximum at 419 nm is not less than 0.35.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *dexamethasone acetate CRS*.

If the spectra obtained in the solid state show differences, record new spectra using saturated solutions (about 30 g/l) in *chloroform R* in a 0.2 mm cell.

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

Solvent mixture: *methanol R, methylene chloride R* (1:9 *V/V*).

Test solution. Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (a). Dissolve 20 mg of *dexamethasone acetate CRS* in the solvent and dilute to 20 ml with the solvent mixture.

Reference solution (b). Dissolve 10 mg of *cortisone acetate R* in reference solution (a) and dilute to 10 ml with reference solution (a).

Plate: *TLC silica gel F_{254} plate R*.

Mobile phase: add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*.

Application: 5 µl.

Development: over a path of 15 cm.

Drying: in air.

Detection A: examine in ultraviolet light at 254 nm.

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

Detection B: spray with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

Results B: the principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability: reference solution (b):

– the chromatogram shows 2 clearly separated spots.

D. Add about 2 mg to 2 ml of *sulphuric acid R* and shake to dissolve. Within 5 min, a faint reddish-brown colour develops. Add this solution to 10 ml of *water R* and mix. The colour is discharged and a clear solution remains.

E. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, add 1 ml of *water R*, 0.05 ml of *phenolphthalein solution R1* and about 1 ml of *dilute hydrochloric acid R* to render the solution colourless. Filter. To a freshly prepared mixture of 0.1 ml of *alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R*, add 1.0 ml of the filtrate. Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The test solution is yellow and the blank is red.

F. About 10 mg gives the reaction of acetyl (*2.3.1*).

TESTS

Specific optical rotation (*2.2.7*): + 84 to + 90 (dried substance).

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

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 25.0 mg of the substance to be examined in about 4 ml of *acetonitrile R* and dilute to 10.0 ml with *water R*.

Reference solution (a). Dissolve 2 mg of *dexamethasone acetate CRS* and 2 mg of *betamethasone acetate CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 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: in a 1000 ml volumetric flask mix 380 ml of *acetonitrile R* with 550 ml of *water R* and allow to equilibrate; dilute to 1000 ml with *water R* and mix again.

Flow rate: 1 ml/min.

Detection: spectrophotometer at 254 nm.

Equilibration: with the mobile phase for about 30 min.

Injection: 20 µl.

Run time: 1.5 times the retention time of dexamethasone acetate.

Retention time: betamethasone acetate = about 19 min; dexamethasone acetate = about 22 min.

System suitability: reference solution (a):

– *resolution*: minimum 3.3 between the peaks due to betamethasone acetate; if necessary, adjust the concentration of acetonitrile in the mobile phase.

Limits:

– *any impurity*: for each impurity, not more than 0.5 times the area of the principal in the chromatogram obtained with reference solution (b) (0.5 per cent);

– *total*: not more than the area of the principal in the chromatogram obtained with reference solution (b) (1.0 per cent);

– *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 0.500 g by drying *in vacuo* in an oven at 105 °C.

ASSAY

Dissolve 0.100 g in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol (96 per cent) R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 238.5 nm.

Calculate the content of $C_{24}H_{31}FO_6$ taking the specific absorbance to be 357.

STORAGE

Protected from light.

01/2008:2237

DEXAMETHASONE ISONICOTINATE

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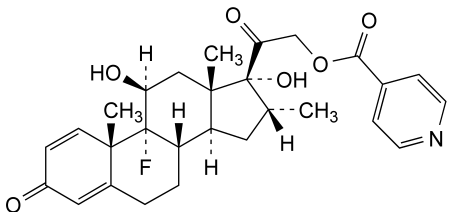

$C_{28}H_{32}FNO_6$ M_r 497.6

[2265-64-7]

DEFINITION

9-Fluoro-11β,17-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl pyridine-4-carboxylate.

Content: 99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white crystalline powder.

Solubility: practically insoluble in water, slightly soluble in anhydrous ethanol and in acetone.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *dexamethasone isonicotinate CRS*.

TESTS

Specific optical rotation (*2.2.7*): + 142 to + 146 (dried substance).

Suspend 0.200 g in 4.0 ml of *ethyl acetate R* and dilute to 20.0 ml with *ethanol (96 per cent) R*. Treat in an ultrasonic bath until a clear solution is obtained.

Related substances. Liquid chromatography (*2.2.29*). *Prepare solutions immediately before use.*

Test solution. Suspend 50.0 mg in 7 ml of *acetonitrile R* and dilute to 10.0 ml with *water R*. Treat in an ultrasonic bath until a clear solution is obtained.

Reference solution (a). Suspend 5.0 mg of *dexamethasone CRS* and 5.0 mg of *dexamethasone acetate CRS* in 70 ml of *acetonitrile R*, add 1.0 ml of the test solution and dilute to 100.0 ml with *water R*. Treat in an ultrasonic bath until a clear solution is obtained.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with *water R*.

Reference solution (c). Suspend 5 mg of *dexamethasone isonicotinate for impurity C identification CRS* in 0.7 ml of *acetonitrile R* and dilute to 1 ml with *water R*. Treat in an ultrasonic bath until a clear solution is obtained.

Column:

– *size*: *l* = 0.125 m, Ø = 4.0 mm,

– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

– *mobile phase A*: *water R*,

– *mobile phase B*: *acetonitrile R*,

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	68	32
2 - 20	68 → 50	32 → 50
20 - 25	50 → 68	50 → 32
25 - 35	68	32

Flow rate: 1.2 ml/min.

Detection: spectrophotometer at 240 nm.

Injection: 10 µl.

Identification of impurities: use the chromatogram supplied with *dexamethasone isonicotinate for impurity C identification CRS* and the chromatogram obtained with reference solution (c) to identify the peak due to impurity C.

Relative retention with reference to dexamethasone isonicotinate (retention time = about 12 min): impurity A = about 0.4; impurity C = about 0.6; impurity B = about 0.8.

System suitability: reference solution (a):

– *resolution*: minimum 5.0 between the peaks due to impurity B and dexamethasone isonicotinate.

Limits:

– *impurity A*: not more than 5 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.5 per cent),

– *impurity B*: not more than 3 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.3 per cent),

– *impurity C*: not more than 3 times the area of the peak due to dexamethasone isonicotinate in the chromatogram obtained with reference solution (b) (0.3 per cent),

– *unspecified impurities*: for each impurity, not more than the area of the peak due to dexamethasone isonicotinate in the chromatogram obtained with reference solution (b) (0.1 per cent),