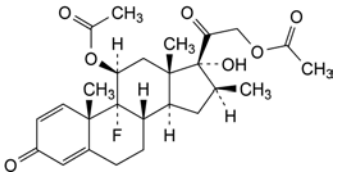
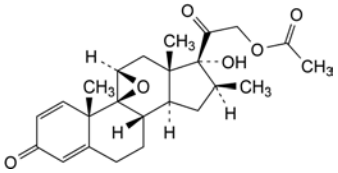


C. 9-fluoro-17-hydroxy-16β-methyl-3,20-dioxopregna-1,4-diene-11β,21-diyl diacetate (betamethasone 11,21-diacetate),

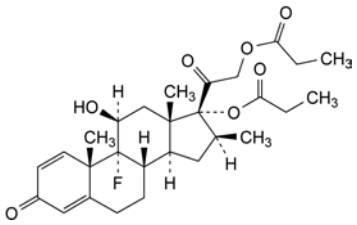


D. 9,11β-epoxy-17-hydroxy-16β-methyl-3,20-dioxo-9β-pregna-1,4-diene-21-yl acetate.

01/2008:0809
corrected 6.0

BETAMETHASONE DIPROPIONATE

Betamethasoni dipropionas



$C_{28}H_{37}FO_7$ M_r 504.6
[5593-20-4]

DEFINITION

9-Fluoro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-diene-17,21-diyl dipropanoate.

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 methylene chloride, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: B, C.

Second identification: A, 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-sulfuric 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 419 nm is not more than 0.10.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *betamethasone dipropionate CRS*.

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 10 mg of *betamethasone dipropionate CRS* in the solvent mixture and dilute to 10 mL with the solvent mixture.

Reference solution (b). Dissolve 10 mg of *desoxycortone acetate CRS* in the solvent mixture and dilute to 10 mL with the solvent mixture. Dilute 5 mL of this solution 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 sulfuric 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. Thin-layer chromatography (*2.2.27*).

Test solution (a). Dissolve 25 mg of the substance to be examined in *methanol R* with gentle heating and dilute to 5 mL with the same solvent (solution A). Dilute 2 mL of this solution to 10 mL with *methylene chloride R*.

Test solution (b). Transfer 2 mL of solution A to a 15 mL glass tube with a ground-glass stopper or a polytetrafluoroethylene cap. Add 10 mL of *saturated methanolic potassium hydrogen carbonate solution R* and immediately pass a current of *nitrogen R* briskly through the solution for 5 min. Stopper the tube. Heat in a water-bath at 45 °C, protected from light, for 2 h. Allow to cool.

Reference solution (a). Dissolve 25 mg of *betamethasone dipropionate CRS* in *methanol R* with gentle heating and dilute to 5 mL with the same solvent (solution B). Dilute 2 mL of this solution to 10 mL with *methylene chloride R*.

Reference solution (b). Transfer 2 mL of solution B to a 15 mL glass tube with a ground-glass stopper or a polytetrafluoroethylene cap. Add 10 mL of *saturated methanolic potassium hydrogen carbonate solution R* and immediately pass a current of *nitrogen R* briskly through the solution for 5 min. Stopper the tube. Heat in a water-bath at 45 °C, protected from light, for 2 h. Allow to cool.

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 each of the chromatograms obtained with the test solutions is similar in position and size to the principal spot in the chromatogram obtained with the corresponding reference solution.

Detection B: spray with *alcoholic solution of sulfuric 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 each of the chromatograms obtained with the test solutions 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

the corresponding reference solution. The principal spot in each of the chromatograms obtained with test solution (b) and reference solution (b) has an R_F value distinctly lower than that of the principal spots in each of the chromatograms obtained with test solution (a) and reference solution (a).

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

F. 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. Add 1.0 mL of the filtrate to a freshly prepared mixture of 0.1 mL of *alizarin S solution R* and 0.1 mL of *zirconyl nitrate solution R*. 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.

TESTS

Specific optical rotation (*2.2.7*): + 63 to + 70 (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 62.5 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 2.5 mg of *betamethasone dipropionate CRS* and 2.5 mg of *anhydrous beclometasone dipropionate CRS* in the mobile phase and dilute to 50.0 mL with the same solvent.

Reference solution (b). Dilute 1.0 mL of the test solution to 50.0 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 carefully 350 mL of *water R* with 600 mL of *acetonitrile 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 45 min.

Injection: 20 µL.

Run time: 2.5 times the retention time of betamethasone dipropionate.

Retention time: betamethasone diproponiate = about 9 min; beclometasone dipropionate = about 10.7 min.

System suitability: reference solution (a):

– *resolution*: minimum 2.5 between the peaks due to betamethasone dipropionate and beclometasone dipropionate; if necessary, adjust the concentration of acetonitrile in the mobile phase.

Limits:

– *any impurity*: for each impurity, not more than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent), and not more than 1 such peak has an area greater than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);

– *total*: not more than 1.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 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).

Loss on drying (*2.2.32*): maximum 1.0 per cent, determined on 0.500 g by drying in an oven at 105 °C.

ASSAY

Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 100.0 mL with the same solvent. Dilute 2.0 mL of this solution to 50.0 mL with *ethanol (96 per cent) R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 240 nm.

Calculate the content of $C_{28}H_{37}FO_7$ taking the specific absorbance to be 305.

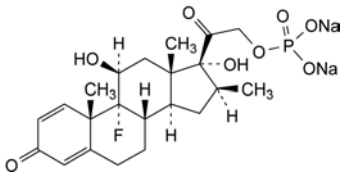
STORAGE

Protected from light.

01/2008:0810

BETAMETHASONE SODIUM PHOSPHATE

Betamethasoni natrii phosphas



$C_{22}H_{28}FNa_2O_8P$ M_r 516.4

[151-73-5]

DEFINITION

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

Content: 96.0 per cent to 103.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white powder, very hygroscopic.

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

IDENTIFICATION

First identification: B, C.

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

A. Dissolve 10.0 mg in 5 mL of *water R* and dilute to 100.0 mL with *anhydrous ethanol R*. Place 2.0 mL of this solution in a ground-glass-stoppered tube, add 10.0 mL of *phenylhydrazine-sulfuric 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 450 nm is not more than 0.10.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *betamethasone sodium phosphate 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 *ethanol (96 per cent) R*, evaporate to dryness on a water-bath and record new spectra using the residues.

C. 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 *betamethasone sodium phosphate CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (b). Dissolve 10 mg of *prednisolone sodium phosphate CRS* in *methanol R* and dilute to 10 mL with the same solvent. Dilute 5 mL of this solution to 10 mL with reference solution (a).

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