TESTS

Specific optical rotation (*2.2.7*): + 96 to + 102 (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 2 mL of *tetrahydrofuran R* and dilute to 10.0 mL with *water R*.

Reference solution (a). Dissolve 2 mg of *prednisolone CRS* and 2 mg of *hydrocortisone CRS* (impurity A) 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*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm);

– *temperature*: 45 °C.

Mobile phase: in a 1000 mL volumetric flask, mix 220 mL of *tetrahydrofuran R* with 700 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; inject the solvent mixture of the test solution as a blank.

Run time: 4.5 times the retention time of prednisolone.

Retention time: prednisolone = about 14 min; impurity A = about 15.5 min.

System suitability: reference solution (a):

– *resolution*: minimum 2.2 between the peaks due to prednisolone and impurity A; if necessary, adjust the concentration of *tetrahydrofuran R* in the mobile phase.

Limits:

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

– *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2 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 1.0 per cent, determined on 0.500 g by drying 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 243.5 nm.

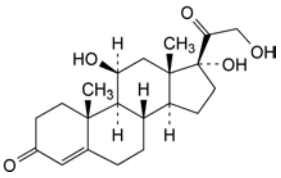
Calculate the content of $C_{21}H_{28}O_5$ taking the specific absorbance to be 415.

STORAGE

In an airtight container, protected from light.

IMPURITIES

Specified impurities: *A*.

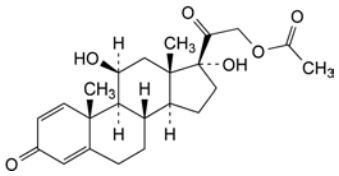


A. 11β,17,21-trihydroxypregn-4-ene-3,20-dione (hydrocortisone).

01/2008:0734
corrected 6.0

PREDNISOLONE ACETATE

Prednisoloni acetas



$C_{23}H_{30}O_6$ M_r 402.5
[52-21-1]

DEFINITION

11β,17-Dihydroxy-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, slightly soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

First identification: A, B.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *prednisolone acetate CRS*.

B. Thin-layer chromatography (*2.2.27*).
Test solution. Dissolve 10 mg of the substance to be examined in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 10 mL with the same mixture of solvents.
Reference solution (a). Dissolve 20 mg of *prednisolone acetate CRS* in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 20 mL with the same mixture of solvents.
Reference solution (b). Dissolve 10 mg of *prednisolone pivalate CRS* in reference solution (a) and dilute to 10 mL with the same solution.
Plate: *TLC silica gel plate F_{254} 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 105 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.
System suitability: reference solution (b):
– the chromatogram obtained shows 2 clearly separated spots.

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).

C. Add about 2 mg to 2 mL of *sulfuric acid R* and shake to dissolve. Within 5 min, an intense red colour develops. When examined in ultraviolet light at 365 nm, a reddish-brown fluorescence is seen. Add the solution to 10 mL of *water R* and mix. The colour fades and there is greenish-yellow fluorescence in ultraviolet light at 365 nm.

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

TESTS

Specific optical rotation (*2.2.7*): + 128 to + 137 (dried substance).

Dissolve 70.0 mg in *methanol R2* and dilute to 20.0 mL with the same solvent.

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

Buffer solution pH 4. Mix 1 volume of *dilute hydrochloric acid R*, 5 volumes of a 68.1 g/L solution of *sodium acetate R*, 15 volumes of a 37.3 g/L solution of *potassium chloride R* and 79 volumes of *water R*.

Solvent mixture. Mix equal volumes of *acetonitrile R* and buffer solution pH 4.

Test solution. Dissolve 25.0 mg of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (a). Dissolve 2 mg of *prednisolone acetate CRS* and 2 mg of *hydrocortisone acetate CRS* (impurity A) in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 2.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c). Dissolve 5 mg of *prednisolone acetate for peak identification CRS* (containing impurities A, B and C) in the solvent mixture and dilute to 50 mL with the solvent mixture.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;
– *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
– *temperature*: 40 °C.

Mobile phase: *acetonitrile R, water R* (350:650 *V/V*).

Flow rate: 1 mL/min.

Detection: spectrophotometer at 254 nm.

Injection: 20 µL.

Run time: 2.5 times the retention time of prednisolone acetate.

Identification of impurities: use the chromatogram supplied with *prednisolone acetate for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B and C.

Relative retention with reference to prednisolone acetate (retention time = about 17 min): impurity B = about 0.4; impurity A = about 1.1; impurity C = about 2.0.

System suitability: reference solution (a):

– *resolution*: minimum 2.0 between the peaks due to prednisolone acetate and impurity A.

Limits:

– *impurities A, B*: for each impurity, not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
– *impurity C*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
– *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
– *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);
– *disregard limit*: 0.25 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 1.000 g by drying 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 243 nm.

Calculate the content of $C_{23}H_{30}O_6$ taking the specific absorbance to be 370.

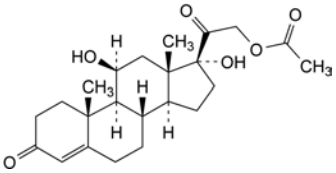
STORAGE

Protected from light.

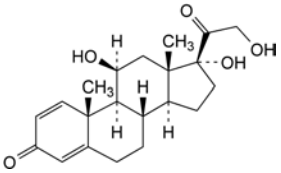
IMPURITIES

Specified impurities: *A, B, C.*

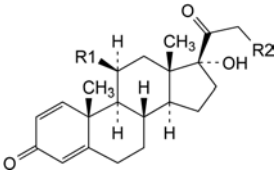
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *D, E.*



A. 11β,17-dihydroxy-3,20-dioxopregn-4-en-21-yl acetate (hydrocortisone acetate),

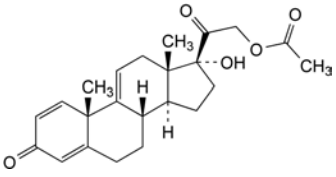


B. 11β,17,21-trihydroxypregna-1,4-diene-3,20-dione (prednisolone),



C. R1 = R2 = O-CO-CH_3: 17-hydroxy-3,20-dioxopregna-1,4-diene-11β,21-diyl diacetate (prednisolone 11,21-diacetate),

D. R1 = OH, R2 = H: 11β,17-dihydroxypregna-1,4-diene-3,20-dione,

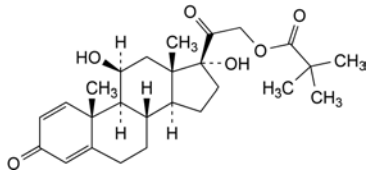


E. 17-hydroxy-3,20-dioxopregna-1,4,9(11)-trien-21-yl acetate.

01/2008:0736
corrected 6.0

PREDNISOLONE PIVALATE

Prednisoloni pivalas



$C_{26}H_{36}O_6$ M_r 444.6
[1107-99-9]

DEFINITION

11β,17-Dihydroxy-3,20-dioxopregna-1,4-dien-21-yl 2,2-dimethylpropanoate.

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

mp: about 229 °C, with decomposition.

IDENTIFICATION

First identification: B, C.

Second identification: A, C, D.

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*) at the absorption maximum at 415 nm is 0.20 to 0.30.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *prednisolone pivalate 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*).

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 *prednisolone pivalate CRS* in the solvent mixture and dilute to 10 mL with the solvent mixture.

Reference solution (b). Dissolve 10 mg of *prednisolone 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, and 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. To 2 mL of *sulfuric acid R*, add about 2 mg and shake to dissolve. Within 5 min, an intense red colour develops. When examined in ultraviolet light at 365 nm, a reddish-brown fluorescence is seen. Add this solution to 10 mL of *water R* and mix. The colour fades and there is greenish-yellow fluorescence in ultraviolet light at 365 nm.

TESTS

Specific optical rotation (*2.2.7*): + 104 to + 112 (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 2 mL of a mixture of 1 volume of *water R* and 4 volumes of *tetrahydrofuran R* and dilute to 25.0 mL with the mobile phase.

Reference solution (a). Dissolve 25 mg of *prednisolone acetate CRS*, 25 mg of *cortisone acetate CRS* and 25 mg of *prednisolone pivalate CRS* in 2 mL of a mixture of 1 volume of *water R* and 4 volumes of *tetrahydrofuran R* and dilute to 25.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 25.0 mL with the mobile phase.

Reference solution (b). Dilute 1.0 mL of the test solution to 50.0 mL with the mobile phase.

Column:

– *size*: *l* = 0.15 m, Ø = 4.6 mm;
– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: carefully mix 19 mL of *butyl acetate R1* with 37 mL of *tetrahydrofuran R* and 213 mL of *ethylene glycol monomethyl ether R*, then add with 231 mL of *water R*; mix, allow to equilibrate for 1 h and filter through a 0.45 µm filter.

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 prednisolone pivalate.

Retention time: prednisolone acetate = about 3.5 min; cortisone acetate = about 4.5 min; prednisolone pivalate = about 13 min.

System suitability: reference solution (a):

– *resolution*: minimum 2.5 between the peaks due to prednisolone acetate and cortisone acetate; if necessary, adjust the concentration of water in the mobile phase.

Limits:

– *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent), and not more than one such peak has an area greater than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 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);

Monographs P