Mobile phase: *acetonitrile R, water R* (5:6 *V/V*).

Flow rate: 0.7 ml/min.

Detection: spectrophotometer at 243 nm.

Injection: 20 µl of the test solution and reference solutions (a) and (b).

Run time: twice the retention time of prednicarbate.

Retention time: prednicarbate = about 17 min; impurity F = about 19 min.

System suitability: reference solution (a):

– *resolution*: minimum 3.0 between the peaks due to prednicarbate and impurity F; if necessary, adjust the composition of the mobile phase.

Limits:

– *impurity F*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);

– *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

– *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent);

– *disregard limit*: 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.0125 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

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: test solution and reference solution (c).

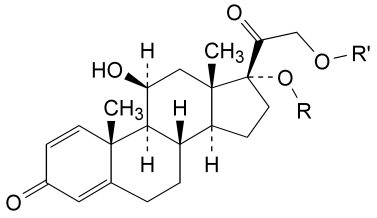
Calculate the percentage content of $C_{27}H_{36}O_8$ from the declared content of *prednicarbate CRS*.

STORAGE

Protected from light.

IMPURITIES

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



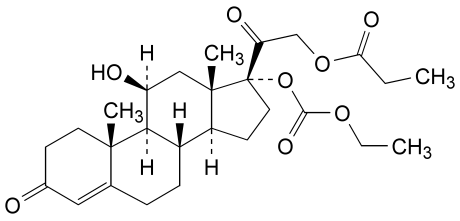
A. R = R′ = H: prednisolone,

B. R = CO-O-C_2H_5, R′ = H: prednisolone 17-ethylcarbonate,

C. R = H, R′ = CO-C_2H_5: prednisolone 21-propanoate,

D. R = H, R′ = CO-O-C_2H_5: prednisolone 21-ethylcarbonate,

E. R = CO-O-C_2H_5, R′ = CO-CH_3: prednisolone 21-acetate 17-ethylcarbonate,



F. 11β-hydroxy-3,20-dioxopregn-4-ene-17,21-diyl 17-ethylcarbonate 21-propanoate(1,2-dihydroprednicarbate).

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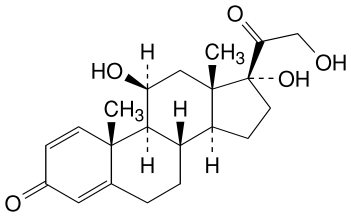
corrected 6.0

PREDNISOLONE

Prednisolonum



$C_{21}H_{28}O_5$ M_r 360.4
[50-24-8]

DEFINITION

11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione.

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

CHARACTERS

Appearance: white or almost white, crystalline, hygroscopic powder.

Solubility: very slightly soluble in water, soluble in ethanol (96 per cent) and in methanol, sparingly soluble in acetone, slightly soluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *prednisolone 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 *acetone R*, evaporate to dryness on a water-bath and record new spectra using the residues.

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

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

Reference solution (a). Dissolve 20 mg of *prednisolone CRS* in the mobile phase and dilute to 20 ml with the mobile phase.

Reference solution (b). Dissolve 10 mg of *hydrocortisone CRS* in reference solution (a) and dilute to 10 ml with reference solution (a).

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

Mobile phase: *methanol R, methylene chloride R* (10:90 *V/V*).

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 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.

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* 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

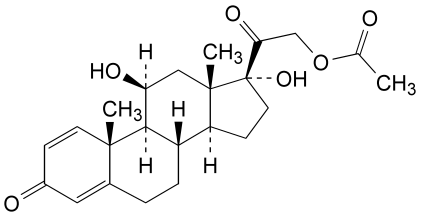
Specified impurities: *A*.

A. 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*.