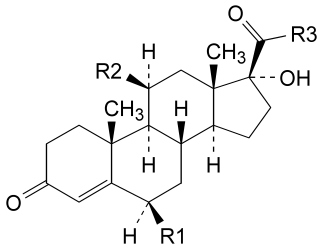
IMPURITIES

A. prednisolone,

B. cortisone,

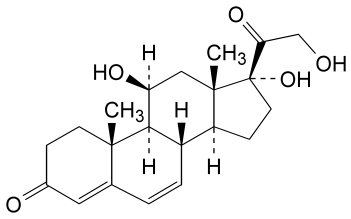
C. hydrocortisone acetate,



D. R1 = R2 = OH, R3 = CH_2OH: 6β,11β,17,21-tetrahydroxypregn-4-ene-3,20-dione (6β-hydroxyhydrocortisone),

F. R1 = R2 = H, R3 = CH_2OH: 17,21-dihydroxypregn-4-ene-3,20-dione (Reichstein's substance),

G. R1 = H, R2 = OH, R3 = CHO: 11β,17-dihydroxy-3,20-dioxopregn-4-en-21-al,

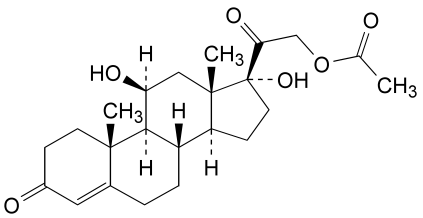


E. 11β,17,21-trihydroxypregna-4,6-diene-3,20-dione (Δ6-hydrocortisone).

01/2008:0334
corrected 6.0

HYDROCORTISONE ACETATE

Hydrocortisoni acetas



$C_{23}H_{32}O_6$ M_r 404.5
[50-03-3]

DEFINITION

11β,17-Dihydroxy-3,20-dioxopregn-4-en-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 anhydrous ethanol and in methylene chloride.

mp: about 220 °C, with decomposition.

IDENTIFICATION

First identification: A, B.

Second identification: C, D, E.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *hydrocortisone acetate CRS.*

B. 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 *hydrocortisone acetate CRS* in the solvent mixture 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 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.

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

Test solution (a). Dissolve 25 mg of the substance to be examined in *methanol R* 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 stream 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 30 min. Allow to cool.

Reference solution (a). Dissolve 25 mg of *hydrocortisone acetate CRS* in *methanol R* 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 stream 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 30 min. 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 sulphuric acid R* and 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 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 spots in the chromatograms obtained with test solution (b) and reference solution (b) have an R_F value distinctly lower than that of the principal spots in the chromatograms obtained with test solution (a) and reference solution (a).

D. Add about 2 mg to 2 ml of *sulphuric acid R* and shake to dissolve. Within 5 min an intense brownish-red colour develops with a green fluorescence which is particularly intense when viewed in ultraviolet light at 365 nm. Add this solution to 10 ml of *water R* and mix. The colour fades and the fluorescence in ultraviolet light does not disappear.

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

TESTS

Specific optical rotation (*2.2.7*): + 158 to + 167 (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 *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a). Dissolve 2 mg of *hydrocortisone acetate CRS* and 2 mg of *cortisone acetate R* in the mobile phase, then 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 400 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: 2.5 times the retention time of hydrocortisone acetate.

Retention time: hydrocortisone acetate = about 10 min; cortisone acetate = about 12 min.

System suitability: reference solution (a):

- *resolution*: minimum 4.2 between the peaks due to hydrocortisone acetate and cortisone acetate; if necessary, adjust the concentration of acetonitrile 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) (1.0 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) (0.5 per cent);
- *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 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 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 241.5 nm.

Calculate the content of $C_{23}H_{32}O_6$ taking the specific absorbance to be 395.

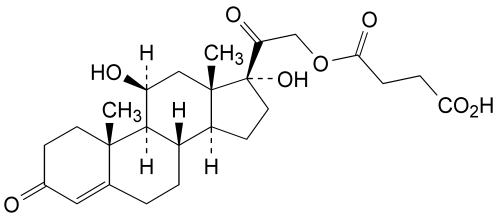
STORAGE

Protected from light.

01/2008:0768
corrected 6.0

HYDROCORTISONE HYDROGEN SUCCINATE

Hydrocortisoni hydrogenosuccinas



$C_{25}H_{34}O_8$ M_r 462.5
[2203-97-6]

DEFINITION

11β,17-Dihydroxy-3,20-dioxopregn-4-en-21-yl hydrogen butanedioate.

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

CHARACTERS

Appearance: white or almost white, hygroscopic powder.

Solubility: practically insoluble in water, freely soluble in acetone and in anhydrous ethanol. It dissolves in dilute solutions of alkali carbonates and alkali hydroxides.