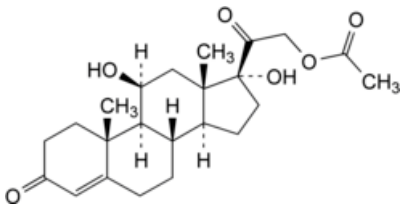
Edition: BP 2025 (Ph. Eur. 11.6 update)

Hydrocortisone Acetate

General Notices

(Ph. Eur. monograph 0334)



$C_{23}H_{32}O_6$ 404.5 *50-03-3*

Action and use

Corticosteroid.

Preparations

Clotrimazole and Hydrocortisone Acetate Cream

Gentamicin and Hydrocortisone Acetate Ear Drops

Hydrocortisone Acetate Cream

Hydrocortisone Acetate and Neomycin Ear Drops

Hydrocortisone Acetate and Neomycin Eye Drops

Hydrocortisone Acetate and Neomycin Eye Ointment

Hydrocortisone Acetate Injection

Hydrocortisone Acetate Ointment

Hydrocortisone Acetate Oral Suspension

Miconazole and Hydrocortisone Acetate Cream

Ph Eur

DEFINITION

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

Content

97.0 per cent to 102.0 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.

IDENTIFICATION

First identification: A, B.

Second identification: C, D, E.

A. Infrared absorption spectrophotometry (2.2.24).

Comparison *hydrocortisone acetate CRS*.

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (d).

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 the 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 the 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 3/4 of the plate.

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* 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 *sulfuric 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*).

Solvent mixture *acetic acid R*, *water R*, *methanol R* (1:10:90 *V/V/V*).

Test solution (a) Dissolve 25.0 mg of the substance to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

Test solution (b) Dilute 1.0 mL of test solution (a) to 10.0 mL with the solvent mixture.

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

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

Reference solution (c) Dissolve 5 mg of *hydrocortisone acetate for peak identification CRS* (containing impurities A, B, D, E and G) in 5 mL of the solvent mixture.

Reference solution (d) Dissolve 25.0 mg of *hydrocortisone acetate CRS* in the solvent mixture and dilute to 25.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 10.0 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).

Mobile phase Mix 400 mL of *acetonitrile R* with 550 mL of *water for chromatography R* and allow to equilibrate; dilute to 1000 mL with *water for chromatography R* and mix again.

Flow rate 1 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL of test solution (a) and reference solutions (a), (b) and (c).

Run time 4 times the retention time of hydrocortisone acetate.

Identification of impurities Use the chromatogram supplied with *hydrocortisone acetate for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, D, E and G; use the chromatogram obtained with reference solution (a) to identify the peak due to impurity C.

Relative retention With reference to hydrocortisone acetate (retention time = about 10 min): impurity A = about 0.4; impurity B = about 0.7; impurity C = about 0.9; impurity D = about 1.2; impurity G = about 1.8; impurity E = about 2.3.

System suitability Reference solution (a):

— *resolution*: minimum 1.5 between the peaks due to impurity C and hydrocortisone acetate.

Limits:

— *impurity C*: not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.6 per cent);

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

— *impurities B, D, E*: for each impurity, not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *impurity G*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 15 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent);

— *disregard limit*: 0.5 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 vacuo* at 60 °C for 3 h.

ASSAY

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

Injection Test solution (b) and reference solution (d).

Run time 1.5 times the retention time of hydrocortisone acetate.

Retention time Hydrocortisone acetate = about 10 min.

Calculate the percentage content of $C_{23}H_{32}O_6$ taking into account the assigned content of *hydrocortisone acetate CRS*.

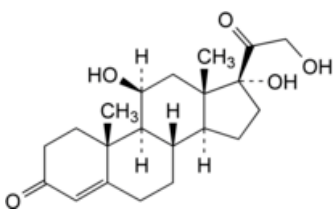
STORAGE

Protected from light.

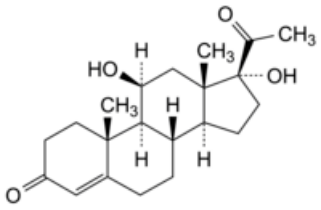
IMPURITIES

Specified impurities *A, B, C, D, E, G.*

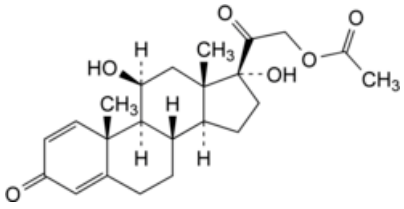
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical 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) *F.*



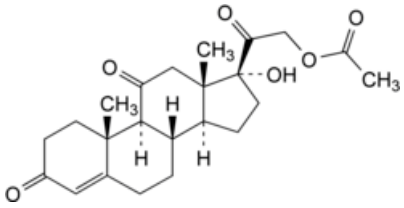
A. 11β,17,21-trihydroxypregn-4-ene-3,20-dione (hydrocortisone),



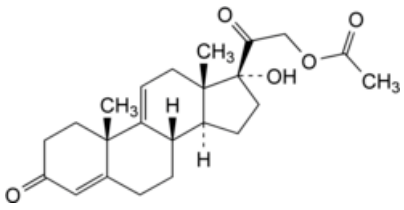
B. 11β,17-dihydroxypregn-4-ene-3,20-dione (oxenol),



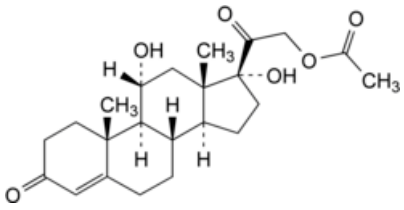
C. 11β,17-dihydroxy-3,20-dioxopregna-1,4-dien-21-yl acetate (prednisolone acetate),



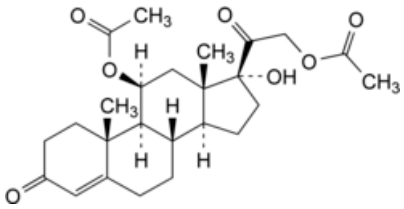
D. 17-hydroxy-3,11,20-trioxopregn-4-en-21-yl acetate (cortisone acetate),



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



F. 11α,17-dihydroxy-3,20-dioxopregn-4-en-21-yl acetate (*epi*-hydrocortisone acetate),



G. 17-hydroxy-3,20-dioxopregn-4-ene-11β,21-diyl diacetate.