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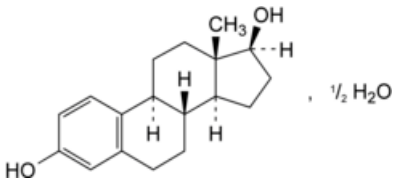
Edition: BP 2025 (Ph. Eur. 11.6 update)

Estradiol Hemihydrate

General Notices

(Ph. Eur. monograph 0821)



$C_{18}H_{24}O_2,½H_2O$ 281.4

Action and use

Estrogen.

Preparations

Estradiol Tablets

Estradiol Transdermal Patches

Estradiol and Norethisterone Tablets

Estradiol and Norethisterone Acetate Tablets

Estradiol Vaginal Tablets

Ph Eur

DEFINITION

Estra-1,3,5(10)-triene-3,17β-diol hemihydrate.

Content

97.0 per cent to 103.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

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Practically insoluble in water, soluble in acetone, sparingly soluble in ethanol (96 per cent), slightly soluble in methylene chloride.

IDENTIFICATION

First identification: B.

Second identification: A, C, D, E.

A. Melting point (2.2.14): 175 °C to 180 °C.
B. Infrared absorption spectrophotometry (2.2.24).

Comparison estradiol hemihydrate CRS.

C. Thin-layer chromatography (2.2.27).

Test solution Dissolve 50 mg of the substance to be examined in methanol R and dilute to 50 mL with the same solvent.

Reference solution (a) Dissolve 50 mg of estradiol hemihydrate CRS in methanol R and dilute to 50 mL with the same solvent.

Reference solution (b) Dissolve 25 mg of ethinylestradiol CRS in reference solution (a) and dilute to 25 mL with reference solution (a).

Plate TLC silica gel plate R.

Mobile phase ethanol (96 per cent) R, toluene R (20:80 *V/V*).

Application 5 µL.

Development Over 3/4 of the plate.

Drying In air until the solvent has evaporated.

Detection Heat at 110 °C for 10 min. Spray the hot plate with alcoholic solution of sulfuric acid R. Heat again at 110 °C for 10 min. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

System suitability The chromatogram obtained with reference solution (b) shows 2 spots which may however not be completely separated.

Results 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).

D. To about 1 mg add 0.5 mL of freshly prepared sulfomolybdic reagent R2. A blue colour develops which in ultraviolet light at 365 nm has an intense green fluorescence. Add 1 mL of sulfuric acid R and 9 mL of water R. The colour becomes pink with a yellowish fluorescence.
E. Water (see Tests).

TESTS

Specific optical rotation (2.2.7)

+ 76.0 to + 83.0 (anhydrous substance).

Dissolve 0.250 g in ethanol (96 per cent) R and dilute to 25.0 mL with the same solvent.

Related substances

Liquid chromatography (2.2.29).

Test solution Dissolve 25 mg of the substance to be examined in 10 mL of acetonitrile R and dilute to 25.0 mL with methanol R2.

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Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 2.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Mix equal volumes of a 1 mg/mL solution of the substance to be examined in *methanol R2* and of a 1 mg/mL solution of *2,3-dichloro-5,6-dicyanobenzoquinone R* in *methanol R2*. Allow to stand for 30 min before injection.

Reference solution (c) Dissolve 5 mg of *estradiol for peak identification CRS* (containing impurities A, B and C) in 2 mL of *acetonitrile R* and dilute to 5.0 mL with *methanol R2*.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase To 400 mL of *acetonitrile R* add 50 mL of *methanol R2* and 400 mL of *water for chromatography R*; allow to stand for 10 min, dilute to 1000 mL with *water for chromatography R* and mix again.

Flow rate 1 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µL.

Run time Twice the retention time of estradiol.

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

Relative retention With reference to estradiol (retention time = about 13 min): impurity D = about 0.9; impurity B = about 1.1; impurity A = about 1.4; impurity C = about 1.9.

System suitability Reference solution (c):

— *resolution*: minimum 2.5 between the peaks due to estradiol and impurity B.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity D by 0.4;

— *impurities A, B, C, D*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 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 (a) (0.10 per cent);

— *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)

2.9 per cent to 3.5 per cent, determined on 0.500 g.

ASSAY

Dissolve 20.0 mg in *ethanol (96 per cent) R* and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of the solution to 50.0 mL with *0.1 M sodium hydroxide*. Allow to cool to room temperature. Measure the absorbance (*2.2.25*) of the solution at the maximum at 238 nm.

Calculate the content of $C_{18}H_{24}O_2$ taking the specific absorbance to be 335.

IMPURITIES

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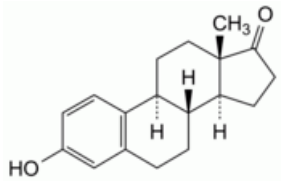
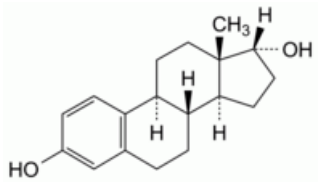
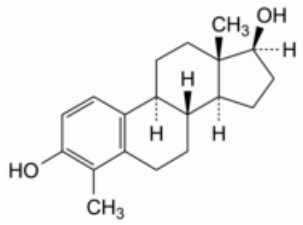
Specified impurities *A, B, C, D.*



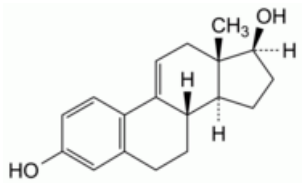
A. 3-hydroxyestra-1,3,5(10)-trien-17-one (estrone),



B. estra-1,3,5(10)-triene-3,17α-diol (17α-estradiol),



C. 4-methylestra-1,3,5(10)-triene-3,17β-diol,



D. estra-1,3,5(10),9(11)-tetraene-3,17β-diol.

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