– *impurities B, E, G*: for each impurity, not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
– *impurity A*: not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
– *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
– *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
– *disregard limit*: 0.1 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 for 3 h.

ASSAY

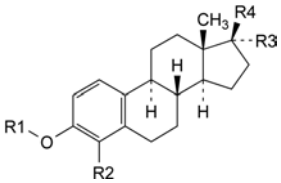
Dissolve 25.0 mg in *anhydrous ethanol R* and dilute to 250.0 mL with the same solvent. Dilute 10.0 mL of this solution to 100.0 mL with *anhydrous ethanol R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 231 nm.

Calculate the content of $C_{25}H_{28}O_3$ taking the specific absorbance to be 500.

IMPURITIES

Specified impurities: A, B, C, 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*): *D, F, H.*



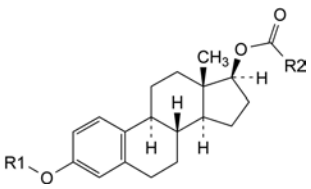
A. R1 = R2 = R3 = H, R4 = OH: estradiol,

B. R1 = CO-C_6H_5, R2 = CH_3, R3 = H, R4 = OH: 17β-hydroxy-4-methylestra-1,3,5(10)-trien-3-yl benzoate,

C. R1 = CO-C_6H_5, R2 = R3 = H, R4 = O-CO-C_6H_5: estra-1,3,5(10)-triene-3,17β-diyl dibenzoate,

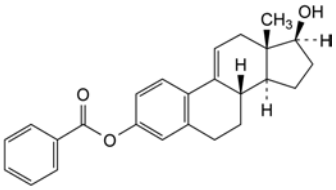
E. R1 = CO-C_6H_5, R2 = R4 = H, R3 = OH: 17α-hydroxyestra-1,3,5(10)-trien-3-yl benzoate,

G. R1 = CO-C_6H_5, R2 = H, R3 + R4 = O: 17-oxoestra-1,3,5(10)-trien-3-yl benzoate (estrone benzoate),



D. R1 = H, R2 = C_6H_5: 3-hydroxyestra-1,3,5(10)-trien-17β-yl benzoate,

H. R1 = CO-C_6H_5, R2 = CH_3: estra-1,3,5(10)-triene-3,17β-diyl 17-acetate 3-benzoate,



F. 17β-hydroxyestra-1,3,5(10),9(11)-tetraen-3-yl benzoate.

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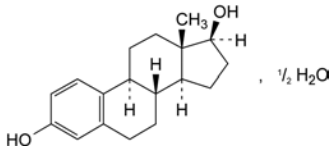
ESTRADIOL HEMIHYDRATE

Estradiolum hemihydricum



$C_{18}H_{24}O_2,{}^1/_2H_2O$ M_r 281.4

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: 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 25.0 mg of the substance to be examined in 10 mL of *acetonitrile R* and dilute to 25.0 mL with *methanol R2*.

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

Reference solution (b). Dissolve 2 mg of *17α-estradiol R* in 5.0 mL of *acetonitrile R*. Mix 2.0 mL of this solution with 1.0 mL of the test solution and dilute to 5.0 mL with the mobile phase.

Reference solution (c). 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 (d). Dissolve 5 mg of *estradiol for peak identification CRS* (estradiol hemihydrate spiked with impurities A, B and C at about 0.5 per cent) in 2 mL of *acetonitrile R* and dilute to 5 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 R*; allow to stand for 10 min, dilute to 1000 mL with *water R* and mix again.

Flow rate: 1 mL/min.

Detection: spectrophotometer at 280 nm.

Equilibration: about 60 min.

Injection: 20 μL.

Run time: twice the retention time of the principal peak.

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

– *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 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 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.

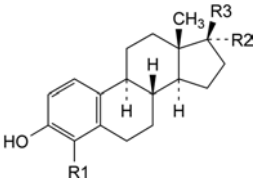
ASSAY

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

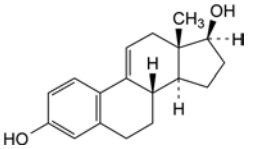
Specified impurities: A, B, C, D.



A. R1 = H, R2 + R3 = O: 3-hydroxyestra-1,3,5(10)-trien-17-one (estrone),

B. R1 = R3 = H, R2 = OH: estra-1,3,5(10)-triene-3,17α-diol (17α-estradiol),

C. R1 = CH_3, R2 = H, R3 = OH: 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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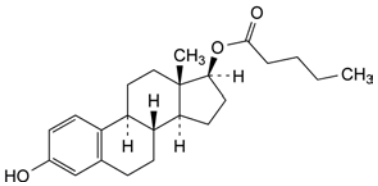
corrected 6.0

ESTRADIOL VALERATE

Estradioli valeras



$C_{23}H_{32}O_3$ M_r 356.5
[979-32-8]

DEFINITION

3-Hydroxyestra-1,3,5(10)-trien-17β-yl pentanoate.

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

CHARACTERS

Appearance: white or almost white, crystalline powder or colourless crystals.

Solubility: practically insoluble in water, soluble in alcohol.

mp: about 145 °C.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *estradiol valerate CRS*.