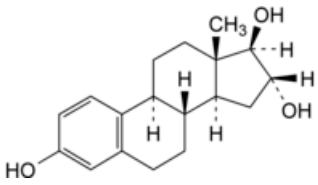
Edition: BP 2025 (Ph. Eur. 11.6 update)

Estriol

General Notices

(Ph. Eur. monograph 1203)



$C_{18}H_{24}O_3$ 288.4 *50-27-1*

Action and use

Estrogen.

Preparation

Estriol Cream

Ph Eur

DEFINITION

Estra-1,3,5(10)-triene-3,16α,17β-triol.

Content

97.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, sparingly soluble in ethanol (96 per cent), practically insoluble in dichloromethane.

IDENTIFICATION

First identification: A, B.

Second identification: C.

A. Infrared absorption spectrophotometry (2.2.24).

Comparison estriol 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 (c).

C. Thin-layer chromatography (2.2.27).

Test solution Dissolve 5 mg of the substance to be examined in methanol R and dilute to 5 mL with the same solvent.

Reference solution Dissolve 5 mg of estriol CRS in methanol R and dilute to 5 mL with the same solvent.

Plate TLC silica gel F_{254} plate R.

Mobile phase 2-propanol R, toluene R (10:90 *V/V*).

Application 5 µL.

Development Over 3/4 of the plate.

Drying In air.

Detection Spray with alcoholic solution of sulfuric acid R. Heat the plate at 120 °C until the spots clearly develop and allow to cool. Examine in daylight and in ultraviolet light at 366 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 366 nm and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Specific optical rotation (2.2.7)

+ 60 to + 65 (dried substance).

Dissolve 80 mg in anhydrous ethanol R and dilute to 10.0 mL with the same solvent.

Related substances

Liquid chromatography (2.2.29).

Solvent mixture methanol R, water R (50:50 *V/V*).

Test solution (a) Dissolve 25.0 mg of the substance to be examined in 25 mL of methanol R and dilute to 50.0 mL with water R.

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

Reference solution (a) Dissolve 5 mg of estriol for system suitability CRS (containing impurities A, D, E and F) in 5 mL of methanol R and dilute to 10.0 mL with water R.

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 25.0 mg of estriol CRS in 25 mL of methanol R and dilute to 50.0 mL with water R. Dilute 1.0 mL of the solution to 10.0 mL with the solvent mixture.

Column:

— *size*: *l* = 0.10 m, Ø = 2.1 mm;

— *stationary phase*: *end-capped, charged surface, ethylene-bridged octadecylsilyl silica gel for chromatography (hybrid material) R* (1.7 µm);

— *temperature*: 50 °C.

Mobile phase:

— *mobile phase A*: *phosphoric acid R*, *methanol R1*, *water for chromatography R* (0.01:28:72 *V/V/V*);

— *mobile phase B*: *phosphoric acid R*, *acetonitrile for chromatography R* (0.01:100 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 9	100	0
9 - 23	100 → 57	0 → 43
23 - 28	57	43

Flow rate 0.4 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µL of test solution (a) and reference solutions (a) and (b).

Identification of impurities Use the chromatogram supplied with *estriol for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, D, E and F.

Relative retention With reference to estriol (retention time = about 11 min): impurity A = about 0.95; impurity F = about 1.45; impurity E = about 1.5; impurity D = about 1.8.

System suitability Reference solution (a):

— *peak-to-valley ratio*: minimum 5.0, where H_P = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to estriol.

Calculation of percentage contents:

— *correction factor*: multiply the peak area of impurity A by 0.5;

— for each impurity, use the concentration of estriol in reference solution (b).

Limits:

— *impurity F*: maximum 0.5 per cent;

— *impurity E*: maximum 0.3 per cent;

— *impurities A, D*: for each impurity, maximum 0.2 per cent;

— *unspecified impurities*: for each impurity, maximum 0.10 per cent;

— *total*: maximum 1.0 per cent;

— *reporting threshold*: 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

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

Mobile phase:

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	90	10
5 - 5.5	90 → 30	10 → 70
5.5 - 7.5	30	70

Injection 5 µL of test solution (b) and reference solutions (a) and (c).

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity A.

Relative retention With reference to estriol (retention time = about 4 min): impurity A = about 0.9.

System suitability Reference solution (a):

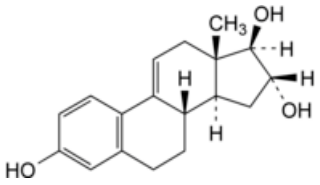
— *peak-to-valley ratio*: minimum 5.0, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to estriol.

Calculate the percentage content of $C_{18}H_{24}O_3$ taking into account the assigned content of *estriol CRS*.

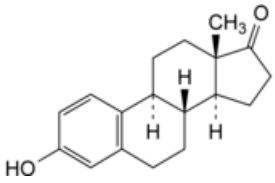
IMPURITIES

Specified impurities *A, D, E, F.*

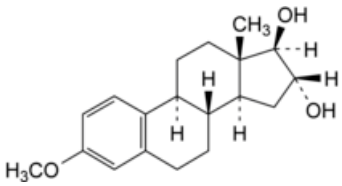
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical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



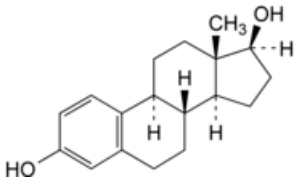
A. estra-1,3,5(10),9(11)-tetraene-3,16α,17β-triol (9,11-didehydroestriol),



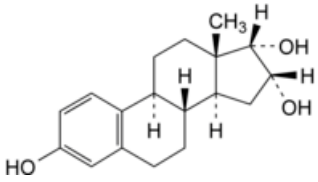
B. 3-hydroxyestra-1,3,5(10)-trien-17-one (estrone),



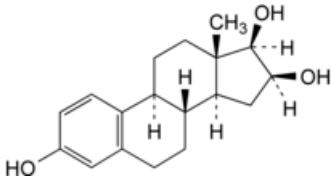
C. 3-methoxyestra-1,3,5(10)-triene-16α,17β-diol (estriol 3-methyl ether),



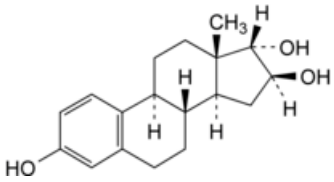
D. estra-1,3,5(10)-triene-3,17β-diol (estradiol),



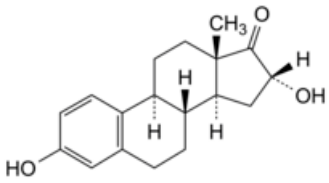
E. estra-1,3,5(10)-triene-3,16α,17α-triol (17-*epi*-estriol),



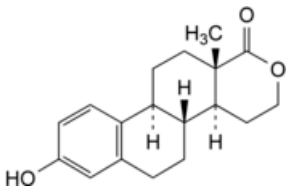
F. estra-1,3,5(10)-triene-3,16β,17β-triol (16-*epi*-estriol),



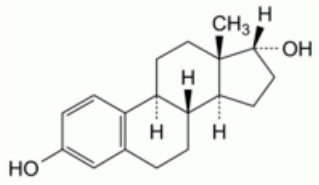
G. estra-1,3,5(10)-triene-3,16β,17α-triol (16,17-*epi*-estriol),



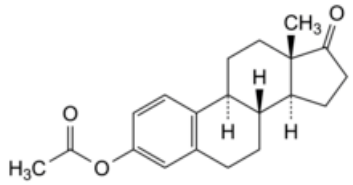
H. 3,16α-dihydroxyestra-1,3,5(10)-trien-17-one,



I. 3-hydroxy-17-oxa-17a-homoestra-1,3,5(10)-trien-17a-one,



J. estra-1,3,5(10)-triene-3,17α-diol (17-*epi*-estradiol),



K. 17-oxoestra-1,3,5(10)-trien-3-yl acetate (estrone acetate).

Ph Eur