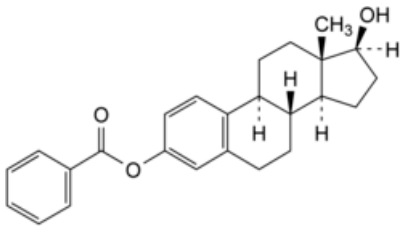
Edition: BP 2025 (Ph. Eur. 11.6 update)

Estradiol Benzoate

General Notices

(Ph. Eur. monograph 0139)



$C_{25}H_{28}O_3$ 376.5 *50-50-0*

Action and use

Estrogen.

Ph Eur

DEFINITION

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

Content

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

CHARACTERS

Appearance

Almost white, crystalline powder or colourless crystals.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in acetone, slightly soluble in methanol.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (2.2.24).

Comparison estradiol benzoate CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in acetone R, evaporate to dryness and record new spectra using the residues.

TESTS

Specific optical rotation (2.2.7)

+ 55.0 to + 59.0 (dried substance).

Dissolve 0.250 g in acetone R and dilute to 25.0 mL with the same solvent.

Related substances

Liquid chromatography (2.2.29).

Test solution Dissolve 20 mg of the substance to be examined in acetonitrile R1 and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dissolve 5 mg of estradiol benzoate for system suitability CRS (containing impurities A, B, C, E and G) in acetonitrile R1 and dilute to 2.5 mL with the same solvent.

Reference solution (b) Dilute 0.5 mL of the test solution to 100.0 mL with acetonitrile R1.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

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

Mobile phase:

— *mobile phase A*: water R, acetonitrile R1 (40:60 *V/V*);

— *mobile phase B*: acetonitrile R1;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	100	0
20 - 21	100 → 10	0 → 90
21 - 31	10	90

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µL.

Identification of impurities Use the chromatogram supplied with estradiol benzoate for system suitability CRS and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, E and G.

Relative retention With reference to estradiol benzoate (retention time = about 19 min): impurity A = about 0.3; impurity E = about 1.1; impurity B = about 1.2; impurity G = about 1.3; impurity C = about 1.5.

System suitability Reference solution (a):

— peak-to-valley ratio: minimum 2.0, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to estradiol benzoate.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 3.3; impurity C = 0.7;

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

— *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

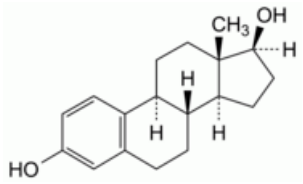
Dissolve 25.0 mg in *anhydrous ethanol R* and dilute to 250.0 mL with the same solvent. Dilute 10.0 mL of this solution to 100.0 mL with *anhydrous ethanol R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 231 nm.

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

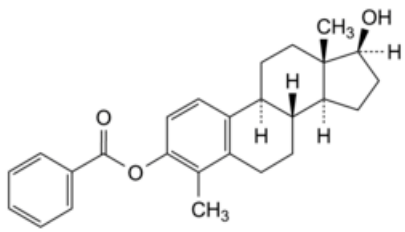
IMPURITIES

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

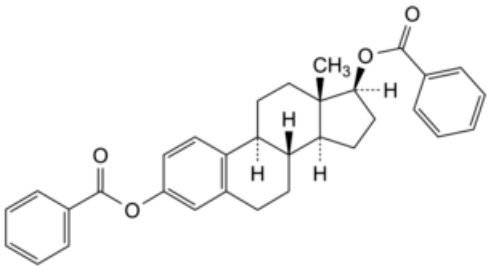
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical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



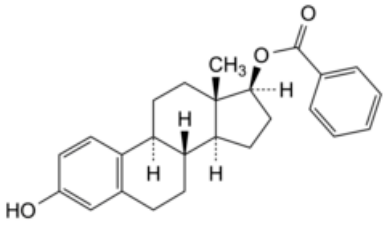
A. estra-1,3,5(10)-triene-3,17β-diol (estradiol),



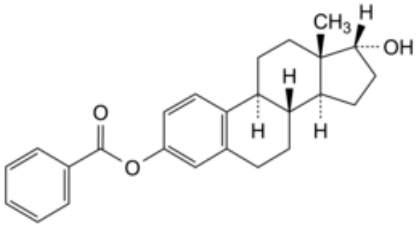
B. 17β-hydroxy-4-methylestra-1,3,5(10)-trien-3-yl benzoate,



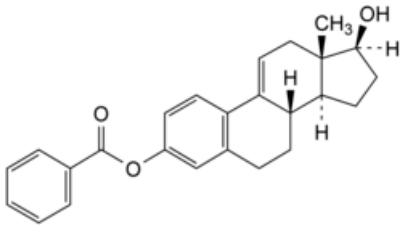
C. estra-1,3,5(10)-triene-3,17β-diyl dibenzoate,



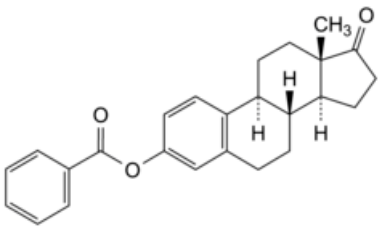
D. 3-hydroxyestra-1,3,5(10)-trien-17β-yl benzoate,



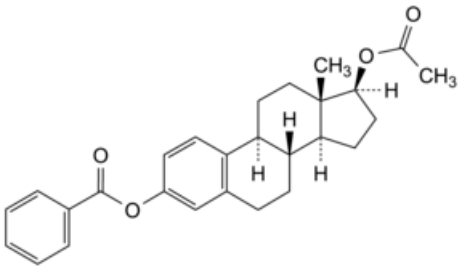
E. 17α-hydroxyestra-1,3,5(10)-trien-3-yl benzoate,



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



G. 17-oxoestra-1,3,5(10)-trien-3-yl benzoate (estrone benzoate),



H. estra-1,3,5(10)-triene-3,17β-diyl 17-acetate 3-benzoate,

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