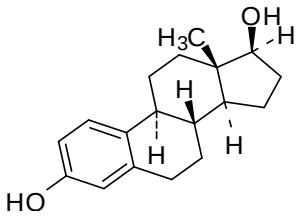
Estradiol Hemihydrate



, 1/2 H_2O

$C_{18}H_{24}O_2$, ½ H_2O Mol. Wt. 281.4

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

Estradiol Hemihydrate contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{18}H_{24}O_2$, ½ H_2O calculated on the anhydrous basis.

Category. Estrogen.

Description. A White or almost white, crystalline powder or colourless crystals.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *estradiol hemihydrate RS* or with the reference spectrum of estradiol hemihydrate.

Test

Specific optical rotation (2.4.22). +76° to +83°, calculated on the anhydrous basis and determined in 1 per cent w/v solution in *ethanol (95 per cent).*

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of *acetonitrile* and dilute to 25.0 ml with the *methanol.*

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). A mixture of equal volumes of 0.1 per cent w/v solutions each of, *estradiol hemihydrate RS* and *2, 3-dichloro-5, 6-dicyanobenzoquinone RS* in *methanol*. Allow to stand for 30 minutes before injection.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with end-capped octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 40 volumes of *acetonitrile*, 55 volumes of *water* and 5 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Name	Relative retention time	Correction factor
Estradiol impurity D[1]	0.9	0.4
Estradiol	1.0	_
Estradiol impurity B[2]	1.1	_

Estradiol impurity A[3]	1.4	_
Estradiol impurity C[4]	1.9	_

[1]estra-1,3,5(10), 9(11)-tetraene-3,17β-diol,
[2]estra-1,3,5(10)-triene-3,17α-diol (17α-estradiol),
[3]3-hydroxyestra-1,3,5(10)-trien-17-one (estrone),
[4]4-methylestra-1,3,5(10)-triene-3,17β-diol.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to estradiol and impurity D is not less than 1.5.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to impurity A, B, C and D, each of, is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (a) (0.1 per cent). The sum of areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with the reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with the reference solution (a) (0.05 per cent).

Water (2.3.43). 2.9 per cent to 3.5 per cent, determined on 0.5 g.

Assay. Dissolve 20.0 mg of substance under examination in *ethanol (95 per cent)* 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 and measure the absorbance of the resulting solution at the maximum at about 238 nm (2.4.7).

Calculate the content of $C_{18}H_{24}O_2$ taking 335 as the specific absorbance at 238 nm.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Solubility. Practically insoluble in *water*, soluble in *acetone*, sparingly soluble in *ethanol (95 per cent)*, slightly soluble in *methylene chloride.*