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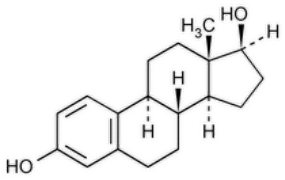
Estradiol



$C_{18}H_{24}O_2$ 272.38

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

Estra-1,3,5(10)-triene-3,17β-diol CAS RN®: 50-28-2; UNII: 4TI98Z838E.

Hemihydrate 281.39 CAS RN®: 35380-71-3; UNII: CXY7B3Q98Z.

» Estradiol contains not less than 97.0 percent and not more than 103.0 percent of $C_{18}H_{24}O_2$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

Labeling—The hemihydrate form is so labeled.

USP Reference Standards ⟨11⟩—

USP Estradiol RS

USP Estrone RS

Identification—

Change to read:

A: ▲*Spectroscopic Identification Tests ⟨197⟩, Infrared Spectroscopy: 197M*▲ *(CN 1-May-2020)* ·

Change to read:

B: ▲*Spectroscopic Identification Tests ⟨197⟩, Ultraviolet-Visible Spectroscopy: 197U*▲ *(CN 1-May-2020)* —

Solution: 50 µg per mL.

Medium: alcohol.

Absorptivities at 280 nm, calculated on the anhydrous basis, do not differ by more than 3.0%.

Melting Range, *Class I* ⟨741⟩: between 173° and 179°. [Note—Dry over silica gel for not less than 16 hours prior to testing.]

Specific Rotation ⟨781S⟩: between +76° and +83°.

Test solution: 10 mg per mL, in dioxane.

Water Determination, *Method I* ⟨921⟩ : not more than 3.5%.

Chromatographic purity—[Note—Make all solutions fresh daily.]

Mobile phase—Prepare a filtered and degassed mixture of 2,2,4-trimethylpentane, *n*-butyl chloride, and methanol (45:4:1). Make adjustments if necessary (see *System Suitability* under *Chromatography ⟨621⟩*).

Diluting solution—Prepare a filtered and degassed mixture of *n*-butyl chloride and methanol (5:1).

Test solution—Transfer about 70 mg of Estradiol, accurately weighed, to a 10-mL volumetric flask, dissolve in *Diluting solution,* shake vigorously to aid dissolution, dilute with *Diluting solution* to volume, and mix.

Chromatographic system (see *Chromatography ⟨621⟩*)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains packing L3. The flow rate is about 2 mL per minute. Chromatograph the *Test solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between estradiol and any impurity is not less than 1.0; the column efficiency is not less than 800 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Inject a volume (about 10 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Estradiol taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response for each impurity; and r_s is the sum of the responses of all the peaks: not more than 0.5% of any individual impurity is found; and not more than 1.0% of total impurities is found.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and water (55:45). Make adjustments if necessary (see *System Suitability* under *Chromatography ⟨621⟩*).

Internal standard solution—Transfer about 300 mg of ethylparaben to a 500-mL volumetric flask, add methanol to volume, and mix.

Standard preparation—Dissolve accurately weighed quantities of USP Estradiol RS and USP Estrone RS in methanol to obtain a solution containing 0.40 mg and 0.24 mg, respectively, in each mL. Pipet 10 mL of this solution and 5 mL of the *Internal standard solution* into a 200-mL volumetric flask. Add 100 mL of methanol, dilute with water to volume, and mix to obtain a solution having a known concentration of about 20 µg of USP Estradiol RS per mL.

Assay preparation—Transfer about 100 mg of Estradiol, accurately weighed, to a 250-mL volumetric flask, add methanol to volume, and mix. Transfer 10.0 mL of this solution to a 200-mL volumetric flask, add 5.0 mL of *Internal standard solution* and 100 mL of methanol, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 205-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.7 for the internal standard, about 1.3 for estrone, and 1.0 for estradiol; the resolution, *R,* between the analyte and estrone is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{18}H_{24}O_2$ in the portion of Estradiol taken by the formula:

$$5C(R_U/R_S)$$

in which C is the concentration, in µg per mL, of USP Estradiol RS in the *Standard preparation;* and R_U and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
ESTRADIOL	Documentary Standards Support	SM52020 Small Molecules 5

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
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