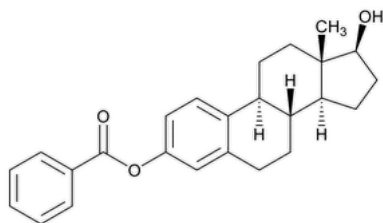


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## Estradiol Benzoate



$C_{25}H_{28}O_3$  376.49  
 Estra-1,3,5(10)-triene-3,17-diol, (17 $\beta$ )-, 3-benzoate;  
 Estradiol 3-benzoate CAS RN<sup>®</sup>: 50-50-0; UNII: 1S4CJB5ZGN.

### DEFINITION

Estradiol Benzoate contains NLT 97.0% and NMT 103.0% of estradiol benzoate ( $C_{25}H_{28}O_3$ ), calculated on the dried basis.

### IDENTIFICATION

#### Change to read:

- **A.** ▲ **SPECTROSCOPIC IDENTIFICATION TESTS (197), Infrared Spectroscopy:** 197A or 197K. ▲ (CN 1-May-2020) [NOTE—If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in [acetone](#), evaporate to dryness, and record new spectra using the residues.]
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### PROCEDURE

**Mobile phase:** [Acetonitrile](#) and [water](#) (7:3)

**System suitability solution:** Transfer 20.0 mg each of [USP Estradiol Benzoate RS](#) and estradiol 17-acetate to a 100-mL volumetric flask. Add 70 mL of [acetonitrile](#), and sonicate until dissolved. Add 25 mL of [water](#), mix well, and allow to equilibrate to ambient temperature. Dilute with [water](#) to volume.

**Standard solution:** Transfer 20.0 mg of [USP Estradiol Benzoate RS](#) to a 100-mL volumetric flask. Add 70 mL of [acetonitrile](#), and sonicate until dissolved. Add 25 mL of [water](#), mix well, and allow to equilibrate to ambient temperature. Dilute with water to volume.

**Sample solution:** Transfer 20.0 mg of Estradiol Benzoate to a 100-mL volumetric flask. Add 70 mL of [acetonitrile](#), and sonicate until dissolved. Add 25 mL of [water](#), mix well, and allow to equilibrate to ambient temperature. Dilute with water to volume.

#### Chromatographic system

(See [Chromatography \(621\), System Suitability](#).)

**Mode:** LC

**Detector:** UV 230 nm

#### Columns

**Guard:** 4.6-mm × 4.5-cm; packing [L1](#)

**Analytical:** 4.6-mm × 25-cm; 5- $\mu$ m packing [L1](#)

**Flow rate:** 1.5 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for estradiol 17-acetate and estradiol benzoate are about 0.5 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 6.0 between estradiol 17-acetate and estradiol benzoate, *System suitability solution*

**Tailing factor:** NMT 2.0, *System suitability solution*

**Relative standard deviation:** NMT 1.5%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of estradiol benzoate ( $C_{25}H_{28}O_3$ ) in the portion of Estradiol Benzoate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of [USP Estradiol Benzoate RS](#) in the *Standard solution* (mg/mL)

$C_U$  = concentration of Estradiol Benzoate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 97.0%–103.0% on the dried basis

#### IMPURITIES

• [RESIDUE ON IGNITION \(281\)](#).

**Sample:** 250 mg

**Acceptance criteria:** NMT 0.2%

• **LIMIT OF METHANOL AND DICHLOROMETHANE:** Proceed as directed in [Residual Solvents \(467\)](#).

**Acceptance criteria:** The sum of methanol and methylene chloride is NMT 0.20%.

• **ORGANIC IMPURITIES**

**Diluent:** [Methylene chloride](#) and [alcohol](#) (2:1)

**Standard solution:** 5 mg/mL of [USP Estradiol Benzoate RS](#) in *Diluent*

**Sample solution A:** 5 mg/mL of Estradiol Benzoate in *Diluent*

**Sample solution B:** 0.1 mg/mL of Estradiol Benzoate in *Diluent* from *Sample solution A*

#### Chromatographic system

(See [Chromatography \(621\), General Procedures, Thin-Layer Chromatography](#).)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Developing solvent system:** [Toluene](#) and [ethyl acetate](#) (70:30)

**Spray reagent:** 50 mg/mL of [ammonium molybdate](#) in 10% [sulfuric acid](#)

#### Analysis

**Samples:** *Standard solution*, *Sample solution A*, and *Sample solution B*

To the plate apply 20-μL aliquots of the *Standard solution* and *Sample solution A* and 20-, 15-, 10-, 5-, and 2-μL aliquots of *Sample solution B*. The volumetric series of *Sample solution B* represents 2.0%, 1.5%, 1.0%, 0.5%, and 0.2% of the concentration of Estradiol Benzoate within the *Sample solution A* spot.

Allow the spots to dry, and develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate.

Spray the plate thoroughly with *Spray reagent*, and dry. Heat the plate in a drying oven for 10 min at about 115°.

Calculate the relative retardation factor (relative to estradiol benzoate) of all spots within the lanes for *Sample solution A* and *Sample solution B*.

Determine the percentages of each impurity by comparing the intensity of the impurity spots within *Sample solution A* to those of the main spots obtained from the series of *Sample solution B*, ignoring any impurity peak less intense than the main spots found in the *Sample solution B* lane containing 0.2% of the amount of estradiol benzoate of *Sample solution A*.

**Acceptance criteria:** See [Table 1](#).

**Table 1**

| Name                                | Relative Retardation Factor | Acceptance Criteria, NMT (%) |
|-------------------------------------|-----------------------------|------------------------------|
| Estradiol <sup>a</sup>              | 0.84                        | 1.0                          |
| Estradiol benzoate                  | 1.00                        | —                            |
| 17a-Estradiol benzoate <sup>b</sup> | 1.15                        | 1.0                          |
| Estrone <sup>c</sup>                | 1.21                        | 1.0                          |
| Any individual unspecified impurity | —                           | 1.0                          |
| Total impurities                    | —                           | 2.0                          |

<sup>a</sup> Estra-1,3,5(10)-triene-3,17b-diol.

<sup>b</sup> Estra-1,3,5(10)-triene-3,17a-diol 3-benzoate.

<sup>c</sup> Estra-1,3,5(10)-triene-17-one, 3-hydroxy.

SPECIFIC TESTS

• [OPTICAL ROTATION \(781S\), Procedures, Specific Rotation](#)

**Sample solution:** 10 mg/mL, previously dried in [dioxane](#)

**Acceptance criteria:** +57.0° to +63.0°

• [LOSS ON DRYING \(731\)](#)

**Analysis:** Dry at 100°–105° for 3 h.

**Acceptance criteria:** NMT 0.5%

• [PARTICLE SIZE DISTRIBUTION ESTIMATION BY ANALYTICAL SIEVING \(786\)](#)

**Suspension fluid:** To a mixture of [glycerin](#) and [water](#) (60:40, w/w) add a sufficient quantity of polysorbate 20 to obtain a solution having a concentration of 125 µL of polysorbate 20 per 100 g of solution.

**Sample suspension:** Saturate the *Suspension fluid* by adding 100 mg of fine Estradiol Benzoate per 100 g of *Suspension fluid*, and sonicate for 10 min. Pass the resulting suspension through a nylon filter of 0.45-µm pore size. To the filtrate, add 50 mg of Estradiol Benzoate per mL of the filtered, saturated *Suspension fluid*, and mix on a vortex mixer until dispersed (about 1 min).

**Analysis:** Using a suitable multi-wavelength particle size analyzer,<sup>1</sup> determine the particle size distribution within the *Sample suspension*, analyzing the results in the range of 5–600 µm.

**Acceptance criteria:** NMT 50% of the particles are less than 30 µm, and NLT 90% of the particles are less than 450 µm. The mean diameter of fine grade Estradiol Benzoate is NMT 100 µm, and the mean diameter of coarse grade Estradiol Benzoate is 100–200 µm.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **LABELING:** Label it to indicate that it is for veterinary use only. Label it to indicate whether it is coarse grade or fine grade.

• [USP REFERENCE STANDARDS \(11\)](#)

[USP Estradiol Benzoate RS](#)

<sup>1</sup> A suitable multi-wavelength particle size analyzer is model LS 13 320, obtained from Beckman Coulter, Inc., Fullerton, CA, or equivalent.

**Auxiliary Information** - Please [check for your question in the FAQs](#) before contacting USP.

| Topic/Question     | Contact                                       | Expert Committee          |
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**Chromatographic Database Information:** [Chromatographic Database](#)

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