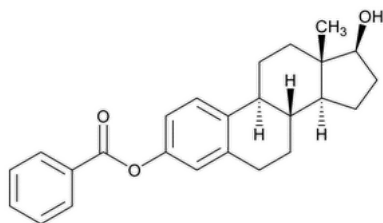


Status: Currently Official on 14-Feb-2025
 Official Date: Official as of 01-May-2020
 Document Type: USP Monographs
 DocId: GUID-4025639B-EE33-4DCE-A4DF-954F0B43DEB2_5_en-US
 DOI: https://doi.org/10.31003/USPNF_M30695_05_01
 DOI Ref: c1lvy

© 2025 USPC
 Do not distribute

Estradiol Benzoate



$C_{25}H_{28}O_3$ 376.49
 Estra-1,3,5(10)-triene-3,17-diol, (17 β)-, 3-benzoate;
 Estradiol 3-benzoate CAS RN[®]: 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- μ m packing [L1](#)

Flow rate: 1.5 mL/min

Injection volume: 20 μ 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 ^a	0.84	1.0
Estradiol benzoate	1.00	—
17a-Estradiol benzoate ^b	1.15	1.0
Estrone ^c	1.21	1.0
Any individual unspecified impurity	—	1.0
Total impurities	—	2.0

^a Estra-1,3,5(10)-triene-3,17b-diol.

^b Estra-1,3,5(10)-triene-3,17a-diol 3-benzoate.