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Estradiol Vaginal Cream

DEFINITION

Estradiol Vaginal Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of estradiol ($C_{18}H_{24}O_2$) in a suitable cream base.

IDENTIFICATION

• A. [THIN-LAYER CHROMATOGRAPHY](#)

Standard solution: 0.5 mg/mL of [USP Estradiol RS](#) in chloroform

Sample solution: Transfer a portion of Vaginal Cream, equivalent to 1 mg of estradiol, to a 150-mL beaker. Add 25 mL of acetonitrile, and gently heat to boiling. Boil for 45 s, and cool to room temperature. Add 25 mL of water, and swirl. Filter with the aid of suction. Transfer the filtrate to a 125-mL separator, add 50 mL of chloroform, and shake. Allow the layers to separate, drain the chloroform layer into a flask, and evaporate in a rotary evaporator to dryness. Dissolve the residue in 2 mL of chloroform.

Chromatographic system

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

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 50 μ L. Dry the applications with the aid of a stream of nitrogen.

Developing solvent system: Toluene and acetone (4:1)

Spray reagent: Sulfuric acid and methanol (1:1)

Analysis

Samples: *Standard solution* and *Sample solution*

Spray the plate with a fine mist of *Spray reagent*, then heat the plate for 3–5 min at 90°. Observe the plate under visible light.

Acceptance criteria: The R_f value and color of the principal spot of the *Sample solution* correspond to those of the *Standard solution*.

ASSAY

• PROCEDURE

Mobile phase: Acetonitrile and water (1:1)

Internal standard solution: 60 μ g/mL of dydrogesterone in acetonitrile. Use a freshly prepared solution.

Standard solution: 10 μ g/mL of [USP Estradiol RS](#) and 7.5 μ g/mL of [USP Estrone RS](#), prepared as follows. Transfer 10 mg of [USP Estradiol RS](#) and 7.5 mg of [USP Estrone RS](#) to a 1000-mL volumetric flask. Add 50.0 mL of *Internal standard solution* and 450 mL of acetonitrile, and mix. Dilute with water to volume.

Sample solution: Nominally 10 μ g/mL of estradiol, prepared as follows. Transfer a portion of Vaginal Cream, equivalent to 0.5 mg of estradiol, to a 150-mL beaker. Add 2.5 mL of *Internal standard solution*, 22.5 mL of acetonitrile, and a few boiling chips. Cover with a watch glass, and heat gently until the Vaginal Cream melts, swirling occasionally. Heat to boiling for about 45 s. Allow to cool to room temperature, add 25.0 mL of water, and mix. Pass first through paper and then through a microdisk filter.

Chromatographic system

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

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm $\times$ 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 50 μ L

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for estradiol, estrone, and the internal standard are 1.0, 1.25, and 2.0, respectively.]

Suitability requirements

Resolution: NLT 1.9 between estradiol and estrone

Relative standard deviation: NMT 3.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of estradiol (C₁₈H₂₄O₂) in the portion of Vaginal Cream taken:

Result = (R_U/R_S) × (C_S/C_U) × 100

R_U = peak response ratio of estradiol to the internal standard from the Sample solution

R_S = peak response ratio of estradiol to the internal standard from the Standard solution

C_S = concentration of USP Estradiol RS in the Standard solution (µg/mL)

C_U = nominal concentration of estradiol in the Sample solution (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- MINIMUM FILL (755): Meets the requirements

SPECIFIC TESTS

- MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62): Meets the requirements of the tests for absence of Staphylococcus aureus and Pseudomonas aeruginosa
- pH (791): 3.5–6.5

ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE: Preserve in collapsible tubes or in tight containers.
- USP REFERENCE STANDARDS (11).
USP Estradiol RS
USP Estrone RS

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

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

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. Information currently unavailable

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