Estradiol Vaginal Inserts

DEFINITION

Estradiol Vaginal Inserts contain NLT 90.0% and NMT 110.0% of the labeled amount of estradiol ($C_{18}H_{24}O_2$).

IDENTIFICATION

Change to read:

- **A. THIN–LAYER CHROMATOGRAPHIC IDENTIFICATION TEST ⟨201⟩**

•[NOTE—When a concentration range is given for a solution, the lower concentration is for inserts labeled as containing 0.01 mg of estradiol, and the high value is for inserts labeled as containing 0.025 mg of estradiol.]• (RB 1-Jul-2010)

Adsorbent: Use a suitable, high-performance thin-layer chromatographic plate.

Sample solution: •Place a number of Inserts, equivalent to 0.38 or 0.95 mg of estradiol, into a vessel. Add 50 mL of isopropyl alcohol, and allow to disintegrate by stirring overnight. Centrifuge the suspension. Evaporate an aliquot of 40 mL of the supernatant to dryness, and dissolve the residue in 3 mL of isopropyl alcohol. Evaporate to dryness, reconstitute with 300 µL of absolute alcohol to obtain a solution containing 1.0 or 2.5 mg/mL of estradiol, and centrifuge.• (RB 1-Jul-2010)

Standard solution: 2.5 mg/mL of USP Estradiol RS in absolute alcohol

Application volume: •NLT 5 µL (equivalent to 10 µg of estradiol)• (RB 1-Jul-2010)

Developing solvent system: Chloroform and acetone (9:1)

Analysis: Proceed as directed in the chapter, using the *Developing solvent system* described above.•Develop the chromatogram over a path of a minimum of 8 cm, and allow the plate to air dry.• (RB 1-Jul-2010) Remove the plate, mark the solvent front, and allow solvent evaporation as described in the chapter. Heat at 100° for about 15 min. Allow the plate to cool, and then immerse it in a mixture of absolute alcohol and concentrated sulfuric acid (95:5). Remove it immediately, place the plate on absorbing paper, and allow it to air-dry. Heat the plate at 100° until •the sulfuric acid has evaporated.• (RB 1-Jul-2010) Examine under UV light at λ = 365 nm.

Acceptance criteria: The principal spot obtained from the *Sample solution* has the same color and R_F value as that from the *Standard solution*.

- **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** •[NOTE—When a concentration range is given for a solution, the lower concentration is for inserts labeled as containing 0.01 mg of estradiol, and the high value is for inserts labeled as containing 0.025 mg of estradiol.]• (RB 1-Jul-2010)

Mobile phase: Acetonitrile and water (11:9)

Diluent: Absolute alcohol and water (1:1)

Estrone standard stock solution: •0.1 mg/mL• (RB 1-Jul-2010) of USP Estrone RS in absolute alcohol

Estradiol standard stock solution: 0.25 mg/mL of USP Estradiol RS in absolute alcohol

System suitability solution: •0.6• (RB 1-Jul-2010) and 2.0 µg/mL of USP Estrone RS and USP Estradiol RS in *Diluent* from *Estrone standard stock solution* and *Estradiol standard stock solution*, respectively

Standard solution: •1.0 or 2.5 µg/mL• (RB 1-Jul-2010) of USP Estradiol RS in *Diluent* from *Estradiol standard stock solution*

Sample solution: •1.0 or 2.5 µg/mL• (RB 1-Jul-2010) of estradiol prepared using 10 Inserts in *Diluent*. Stir the mixture overnight with a magnetic stirrer, shake thoroughly, and centrifuge if necessary.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 205 nm
Column: 3.9-mm × 30-cm; 4-µm packing L1
Flow rate: 1 mL/min
Injection size: 20 µL

System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between estradiol and estrone
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{18}H_{24}O_2$ in the portion of Inserts 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 RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of estradiol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

- **DISSOLUTION ⟨711⟩**

Medium: Phosphate buffer pH 4.75 ± 0.05 (100 g of potassium •dihydrogen• (RB 1-Jul-2010) phosphate in 10 L of water, pH adjusted to 4.75 ± 0.05 with 1 N sodium hydroxide); 500 mL

Apparatus 1: 40 rpm

Time: 3, 5, and 10 h

Mobile phase: Methanol, acetonitrile, and water (27.5:27.5:45)

Standard stock solution: 0.1 mg/mL of USP Estradiol RS in absolute alcohol

Standard solutions: •Quantitatively dilute with water the *Standard stock solution* to obtain solutions with final concentrations equal to approximately 20%, 60%, and 160% of the expected concentration of estradiol in the *Medium* for inserts containing 0.025 mg, assuming complete dissolution, and approximately 20%, 50%, 150%, and 400% of the expected concentration of estradiol in the *Medium* for inserts containing 0.01 mg of estradiol, assuming complete dissolution.• (RB 1-Jul-2010)

Sample solutions: Use the solution under test, unfiltered.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Fluorescence
Excitation wavelength: 230 nm
Emission wavelength: 310 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L1; or 4.6-mm × 7.5-cm; •5.0-µm• (RB 1-Jul-2010) packing L1

Flow rate: 1 mL/min
Injection size: 200 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.8
Relative standard deviation: NMT 2%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the amount of $C_{18}H_{24}O_2$ dissolved:

$$\text{Result} = (A_n \times V_n + (\text{RB 1-Jul-2010}) \sum_{1}^{n-1}(\Delta V_{(n-1)} \times A_{(n-1)}))/V$$

A_n = percentage of estradiol, at the sample point n (e.g., A_2 at the second sampling point)
V_n = volume of *Medium* in the vessel before the sample is taken (mL)
$\Delta V_{(n-1)}$ = volume of sample taken at the sampling point (n–1)
$A_{(n-1)}$ = amount of estradiol (uncorrected) at the sample point (n–1)
V = volume of the medium, 500 mL

Tolerances: The percentage of the labeled amount of estradiol dissolved at the specified times conforms to *Acceptance Table 2*.

Time (h)	Amount Dissolved (%)
3	25–50
5	40–80
10	NLT 80

IMPURITIES

Change to read:

Organic Impurities •[NOTE—When a concentration range is given for a solution, the lower concentration is for inserts labeled as containing 0.01 mg of estradiol, and the high value is for inserts labeled as containing 0.025 mg of estradiol.]• (RB 1-Jul-2010)

• **PROCEDURE**
Solution A: Acetonitrile
Solution B: Water
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	16	84
35	68	32

[NOTE—Before the next injection, run the system at the initial condition until equilibration is achieved.]
System suitability solution: 100 µg/mL of USP Estradiol RS, 0.5 µg/mL of USP Estradiol Related Compound B RS, and 0.5 µg/mL of USP Estradiol Related Compound C RS in absolute alcohol
Sample solution: •Place a number of inserts into a measured volume of absolute alcohol to obtain a solution having an estradiol concentration of 2.4 or 6.0 µg/mL. Stir for a minimum of 16 h, shake thoroughly, and centrifuge if necessary.• (RB 1-Jul-2010) Evaporate 10.0 mL of the supernatant to dryness. Dissolve the residue in 1.0 mL of water and add 7.0 mL of a mixture of toluene and acetone (5:2), mix on a whirl mixer, allow to stand for 1 h, and evaporate 5 mL of the organic phase to dryness. The residue is reconstituted in 450 µL of absolute alcohol to obtain a solution containing •38 or 95 µg/mL• (RB 1-Jul-2010) of estradiol. Centrifuge, and use the supernatant as the *Sample solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1 mL/min
Injection size: 25 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for estradiol related compound B and estradiol are about 0.96 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between estradiol related compound B and estradiol
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the Inserts taken:

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

r_U = peak response for each impurity from the *Sample solution*
r_S = peak response for the estradiol peak from the *Sample solution*

Acceptance criteria
Individual impurities: See •*Impurity Tables 1* and *2*.• (RB 1-Jul-2010)
Total impurities: •NMT 4.4% for inserts labeled as containing 0.025 mg of estradiol and NMT 4.0% for inserts labeled as containing 0.01 mg of estradiol• (RB 1-Jul-2010)

Impurity Table 1. •For inserts labeled to contain 0.025 mg of estradiol• (RB 1-Jul-2010)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Estradiol related compound C (6-Ketoestradiol)[a]	•0.71• (RB 1-Jul-2010)	2.4
Estradiol related compound B (6-Dehydroestradiol)[b]	0.96	1.4
Estradiol	1.0	—
Any other individual impurity	—	0.8

[a] 1,3,5(10)-Estratrien-3,17β-diol-6 one.
[b] 1,3,5(10),6-Estratetraen-3,17β-diol.

•Impurity Table 2. For inserts labeled to contain 0.01 mg of estradiol

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Estradiol related compound C (6-Ketoestradiol)[a]	0.71	1.5
Estradiol related compound B (6-Dehydroestradiol)[b]	0.96	1.3

[a] 1,3,5(10)-Estratrien-3,17β-diol-6-one.
[b] 1,3,5(10),6-Estratetraen-3,17β-diol.

Impurity Table 2. For inserts labeled to contain 0.01 mg of estradiol *(Continued)*

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Estradiol	1.0	—
Any other individual impurity	—	1.3

[a] 1,3,5(10)-Estratrien-3,17β-diol-6-one.
[b] 1,3,5(10),6-Estratetraen-3,17β-diol.

● (RB 1-Jul-2010)

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨**61**⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨**62**⟩**:** The total aerobic microbial count does not exceed 100 cfu/g, and the total combined molds and yeasts count does not exceed 10 cfu/g. Inserts meet the requirements of the tests for absence of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in a tight container, and store at controlled room temperature. Do not refrigerate.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Estradiol RS
  USP Estradiol Related Compound B RS
  USP Estradiol Related Compound C RS
  USP Estrone RS