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Estradiol Valerate Injection

DEFINITION

Estradiol Valerate Injection is a sterile solution of Estradiol Valerate in a suitable vegetable oil. It contains NLT 90.0% and NMT 115.0% of the labeled amount of estradiol valerate ($C_{23}H_{32}O_3$).

IDENTIFICATION

• **A.**

Phenol reagent (Folin-Ciocalteu reagent)**:** In a 1500-mL flask connected by a standard taper joint to a reflux condenser, dissolve 100 g of sodium tungstate and 25 g of sodium molybdate in 700 mL of water. Add 50 mL of phosphoric acid and 100 mL of hydrochloric acid, and reflux gently for 10 h. Cool, and add 150 g of lithium sulfate, 50 mL of water, and 4–6 drops of bromine. Boil the mixture without the condenser for 15 min to remove the excess bromine. Cool, transfer to a 1-L volumetric flask, dilute with water to volume, and filter. The filtrate is golden yellow in color, and has no greenish tint. Store the filtrate in a tight container in a refrigerator. Mix the filtrate with water (1:2) before use as the *Phenol reagent*.

Analysis: Transfer 0.5 mL of Injection to a separator containing 10 mL of solvent hexane and 10 mL of 80% methanol. Shake the contents for 2 min, and allow the phases to separate. Add 1 mL of *Phenol reagent* and 3 mL of sodium carbonate solution (1 in 5) to 1 mL of the bottom layer, and mix.

Acceptance criteria: A blue color develops.

ASSAY

• **Procedure**

Mobile phase: Dissolve 0.8 g of ammonium nitrate in 300 mL of water, add 700 mL of acetonitrile, and mix.

Internal standard solution: 8.0 mg/mL of testosterone benzoate in tetrahydrofuran

Standard solution: 0.8 mg/mL of USP Estradiol Valerate RS, prepared as follows. Transfer 20 mg of USP Estradiol Valerate RS to a 25-mL volumetric flask. Add 5.0 mL of the *Internal standard solution*, and dilute with tetrahydrofuran to volume.

Sample solution: Nominally 0.8 mg/mL of estradiol valerate, prepared as follows. Using a "to contain" pipet, transfer an accurately measured volume of Injection, equivalent to 20 mg of estradiol valerate, to a 25-mL volumetric flask. Rinse the pipet with small portions of tetrahydrofuran, collecting the washings in the volumetric flask. Add 5.0 mL of *Internal standard solution*, and dilute with tetrahydrofuran to volume.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[Note—The relative retention times for estradiol valerate and testosterone benzoate are 1.0 and 1.2, respectively.]

Suitability requirements

Resolution: NLT 3.0 between estradiol valerate and the internal standard

Column efficiency: NLT 1100 theoretical plates for estradiol valerate

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of estradiol valerate ($C_{23}H_{32}O_3$) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

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

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

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C_S = concentration of USP Estradiol Valerate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

IMPURITIES

• **Limit of Estradiol**

Standard solution: A solution of estradiol in acetone containing 30.0% of the labeled concentration of the Injection. Dilute 1.0 mL of this solution with oil labeled as vehicle for Injection to 10.0 mL.

Sample solution: Injection

Chromatographic system

(See *Chromatography ⟨621⟩, Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 5 µL

Developing solvent system: Cyclohexane and ethyl acetate (7:3)

Spray reagent: A 3-in-10 solution of methanol in sulfuric acid, prepared as follows. To 30 mL of methanol in a 100-mL volumetric flask in an ice bath, cautiously add sulfuric acid to volume.

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the samples at spots 2.5 cm from the bottom edge of the plate. Allow the applications to be absorbed by the layer without air-drying. Develop the chromatogram in an unlined chamber until the solvent front has moved about 15 cm above the point of application. Remove the plate, dry at 90° for 30 min, and spray the plate lightly with *Spray reagent*. Heat the plate at 90° for 30 min.

Acceptance criteria: NMT 3.0%; any spot from the *Sample solution* close to the origin and corresponding to the estradiol spot is not larger or more intense than that from the *Standard solution*.

SPECIFIC TESTS

• **Other Requirements:** Meets the requirements in *Injections and Implanted Drug Products ⟨1⟩*

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in single-dose or multiple-dose, light-resistant containers, preferably of Type I or Type III glass.

• **USP Reference Standards ⟨11⟩**

USP Estradiol Valerate RS

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

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

Chromatographic Database Information: Chromatographic Database

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