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Ethinyl Estradiol Tablets

DEFINITION
Ethinyl Estradiol Tablets contain NLT 90.0% and NMT 115.0% of the labeled amount of $C_{20}H_{24}O_2$.

IDENTIFICATION

• **Thin-Layer Chromatographic Identification Test ⟨201⟩**

Standard solution: 0.03 mg/mL of USP Ethinyl Estradiol RS in methanol

Sample solution: Transfer 25 Tablets to a suitable container, add 50 mL of water, and sonicate until the Tablets disintegrate (if needed, remove any coating with water before sonication). Place the sample in a separatory funnel, add 25 mL of ether, and shake well to extract the actives. Using a glass pipet, transfer the ether layer to a clean beaker, and evaporate to 10 mL.

Spray reagent: Methanol and sulfuric acid (1:1)

Application volume: 30 µL

Developing solvent system: Chloroform and alcohol (24:1)

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed under *Chromatography ⟨621⟩, Thin-Layer Chromatography*. Spray the plate with *Spray reagent*, place in an oven at 105° for about 5 min, and examine the plate.

Acceptance criteria: Meet the requirements

ASSAY

• **Procedure**

Mobile phase: Acetonitrile and 20 mM potassium phosphate buffer, pH 6.0 (1:1)

Diluent: Acetonitrile and water (1:1)

Standard stock solution: 0.3 mg/mL of USP Ethinyl Estradiol RS in methanol

Standard solution: 0.12 µg/mL of USP Ethinyl Estradiol RS from *Standard stock solution* in *Diluent*

Sample solution: 0.12 µg/mL of ethinyl estradiol from NLT 20 Tablets in *Diluent*. [Note—Shake for about 30 min before makeup to volume. Centrifuge a portion of the solution, and makeup with *Diluent*.]

Chromatographic system

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

Mode: LC

Detector: Spectrofluorometric, with an excitation wavelength of 285 nm and an emission wavelength of 310 nm

Column: 4.6-mm × 15-cm; packing L11

Guard column: 4.6-mm × 12.5-mm; packing L11

Flow rate: 2 mL/min

Injection size: 200 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for ethinyl estradiol

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{20}H_{24}O_2$ in the portion of Tablets taken:

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

r_U = peak response of ethinyl estradiol from the *Sample solution*

r_S = peak response of ethinyl estradiol from the *Standard solution*

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

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

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS

• **Dissolution**

[Note—Care must be taken not to expose any of the solutions to plastic or rubber. Fluorescent material will leach into the solutions and interfere with the quantitation of ethinyl estradiol. Also, adsorption may occur.]

Test 1

Medium: 0.3% sodium lauryl sulfate in water; 500 mL, degassed

Apparatus 2: 100 rpm

Time: 30 min

pH 6.0 phosphate buffer: Transfer 2.7 g of monobasic potassium phosphate to a 1-L volumetric flask. Dissolve in 900 mL of water. Adjust with 1 N sodium hydroxide to a pH of 6.0, and dilute with water to volume.

Mobile phase: *pH 6.0 phosphate buffer* and acetonitrile (1:1)

Standard stock solution: 0.25 mg/mL of USP Ethinyl Estradiol RS in methanol. This solution is stable for 14 days.

Standard solution: Dilute the *Standard stock solution* with *Medium* to a final concentration of 0.06 µg/mL. Add 1 or 2 drops of methanol to dissipate the bubbles, if necessary. This solution is stable for 24 h.

Sample solution: Centrifuge the solution under test for 10 min at 2000 rpm. Use the supernatant.

Chromatographic system

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

Mode: LC

Detector: Fluorescence, excitation at 285 nm, emission at 310 nm

Column: 4.6-mm x 15-cm, 5-µm packing L11

Guard column: 4.6-mm × 1.25-cm, 5-µm packing L11

Flow rate: 2.0 mL/min

Injection size: 200 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis: Calculate the percentage of ethinyl estradiol dissolved:

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

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 500 mL

Tolerances: NLT 80% (Q) of the labeled amount of ethinyl estradiol is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 5 ppm of polysorbate 80 in water; 500 mL, deaerated with helium

Apparatus 2: 75 rpm

Time: 45 min

Standard stock solution: Transfer 10 mg of USP Ethinyl Estradiol RS and 50 mg of USP Norgestrel RS to a 500-mL volumetric flask. Add 250 mL of acetonitrile, and sonicate until dissolved. Cool to room temperature, and dilute with water to volume. The final concentration is about 20 µg/mL of ethinyl estradiol and 100 µg/mL of norgestrel. This solution is stable for 15 days.

Standard solution: Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of 0.02 µg/mL of ethinyl estradiol. This solution is stable for 6 days.

Sample solution: Centrifuge the solution under test at about 3000 rpm for 20 min. Use the supernatant. This solution is stable for 12 h.

Mobile phase: Water, acetonitrile, and methanol (55:40:5)

Chromatographic system

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

Mode: LC

Detector: UV 200 nm

Column: 4.6-mm × 10-cm, 3-µm packing L1

Column temperature: 30°

Flow rate: 1.2 mL/min

Injection size: 200 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 6.0 between the ethinyl estradiol and norgestrel peaks

Relative standard deviation: NMT 3.0%, for ethinyl estradiol

Analysis: Calculate the percentage of ethinyl estradiol dissolved:

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

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 500 mL

Tolerances: NLT 80% (Q) of the labeled amount of ethinyl estradiol is dissolved.

• **Uniformity of Dosage Units:** Meet the requirements

IMPURITIES

Organic Impurities

• **Procedure**

Solution A: Acetonitrile and 20 mM potassium phosphate buffer, pH 6.0 (1:1)

Solution B: Acetonitrile and 20 mM potassium phosphate buffer, pH 6.0 (4:1)

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)	Flow Rate (mL/min)
0	100	0	2
20	100	0	2
20.1	100	0	2.5
25.0	0	100	2.5
25.1	0	100	3
30.0	0	100	3
30.1	0	100	2
32.0	100	0	2

Time (min)	Solution A (%)	Solution B (%)	Flow Rate (mL/min)
35.0	100	0	2

Diluent: Acetonitrile and water (1:1)

Standard stock solution: 0.3 mg/mL of USP Ethinyl Estradiol RS in methanol

Standard solution: 0.12 µg/mL of USP Ethinyl Estradiol RS from *Standard stock solution* in *Diluent*

Sample solution A: Transfer 20 Tablets into a 200-mL volumetric flask. Add 120 mL of *Diluent*, and shake for about 30 min. Dilute with *Diluent* to volume. Centrifuge a portion of the dissolution sample, and use the clear supernatant.

Sample solution B: 0.6 µg/mL of ethinyl estradiol from *Sample solution A* in *Diluent*

Chromatographic system

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

Mode: LC

Detectors: UV detector at 210 nm; and a spectrofluorometric detector with an excitation wavelength of 285 nm and an emission wavelength of 310 nm

Column: 4.6-mm × 15-cm; packing L11

Guard column: 4.6-mm × 12.5-mm; packing L11

Flow rate: See the gradient table above.

Injection size: 200 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for ethinyl estradiol

Relative standard deviation: NMT 2.0%

Analysis

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

[NOTE—Measure the peak heights for the major peaks obtained within 20 min. Use the response from *Sample solution A* for estrone and all other impurities. Use *Sample solution B* for 17β-ethinyl estradiol.]

Calculate the percentage of 17β-ethinyl estradiol in the portion of Tablets taken:

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

r_U = height of any peak at the relative retention time of 1.16, using the spectrofluorometric detector

r_S = peak height of ethinyl estradiol, using the spectrofluorometric detector

Calculate the percentage of estrone in the portion of Tablets taken:

$$\text{Result} = [(r_U/r_S) \times 100] - E$$

r_U = height of any peak at the relative retention time of 1.2, using the UV detector at 210 nm

r_S = peak height of ethinyl estradiol, using the UV detector at 210 nm

E = percentage of 17β-ethinyl estradiol

Calculate the percentage of any other impurity:

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

r_U = height of any peak other than those mentioned above

r_S = peak height of ethinyl estradiol, using the UV detector at 210 nm

Acceptance criteria

17β-Ethinyl estradiol: NMT 0.5%

Estrone: NMT 0.5%

Any other impurity: NMT 0.5%

Total impurities: NMT 2.0 %

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.
- **Labeling:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP Reference Standards** ⟨11⟩

  USP Ethinyl Estradiol RS

  USP Norgestrel RS

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

Topic/Question	Contact	Expert Committee
ETHINYL ESTRADIOL TABLETS	Documentary Standards Support	SM52020 Small Molecules 5

Chromatographic Database Information: Chromatographic Database

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