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Levonorgestrel and Ethinyl Estradiol Tablets

DEFINITION

Levonorgestrel and Ethinyl Estradiol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of levonorgestrel ($C_{21}H_{28}O_2$) and NLT 90.0% and NMT 110.0% of the labeled amount of ethinyl estradiol ($C_{20}H_{24}O_2$).

IDENTIFICATION

• **A.** The retention times of the two major peaks of the *Sample solution* correspond to those of levonorgestrel and ethinyl estradiol in the *Standard solution*, as obtained in the *Assay*.

• **B.** Finely powder 20 Tablets and transfer a portion of the powder, equivalent to 4 mg of levonorgestrel, to a suitable container. Add 250 mL of a solvent mixture consisting of isooctane and chloroform (3:1). Sonicate the mixture for 3 min, and then stir it by mechanical means for 30 min. Filter the mixture and evaporate the filtrate to dryness in a rotating vacuum evaporator. Dissolve the residue in 3 mL of chloroform, and transfer with a pipet to a 60-mL separator containing 18 mL of isooctane. Rinse the evaporator flask with an additional 3-mL portion of chloroform, and add the rinsing to the separator. Add 10 mL of 1 N sodium hydroxide, shake vigorously, and allow the layers to separate. Discard the lower aqueous phase, and filter the organic phase through 3 g of anhydrous sodium sulfate on filter paper into a 50-mL beaker. Rinse the filter with several small portions of the mixture of isooctane and chloroform (3:1), adding the filtered rinsings to the filtrate, and evaporate under nitrogen on a steam bath to dryness. Dissolve the residue in 1–2 mL of hot toluene, and transfer with a pipet to a small glass vial. Reduce the volume of the solution to 0.1 mL under nitrogen with warming. [Note—During this step, any crystals that deposit on the vial wall should be transferred to the bottom, and allowed to redissolve.]

Store the vial containing the clear toluene solution at 4° overnight to allow crystallization to occur. Remove and discard the mother liquor with a pipet, rinse the crystals with two 0.5-mL portions of anhydrous ether, and discard the rinsings. Dry the vial containing the rinsed crystals in a vacuum desiccator at 60° for 4 h.

Acceptance criteria: The melting point of the dried crystals of levonorgestrel so obtained is not lower than 220°, using the procedure described under *Melting Range or Temperature ⟨741⟩, Class I*.

ASSAY

• **Procedure**

Mobile phase: Acetonitrile, methanol, and water (35:15:45)

Standard solution: 15 µg/mL of USP Levonorgestrel RS and 3 µg/mL of USP Ethinyl Estradiol RS in *Mobile phase*

Sample solution: Transfer a number of Tablets, equivalent to 3 mg of levonorgestrel, to a 200-mL volumetric flask. Dilute with *Mobile phase* to volume, sonicate to disintegrate the Tablets, then shake by mechanical means for 20 min. Centrifuge, and use the clear supernatant.

Chromatographic system

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

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 5- to 7-µm packing L7

Flow rate: 1 mL/min

Injection size: 50 µL

System suitability

Sample: *Standard solution*

[Note—The relative retention times for ethinyl estradiol and levonorgestrel are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.5 between the two major peaks

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{21}H_{28}O_2$ and $C_{20}H_{24}O_2$ in the portion of Tablets taken:

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

r_U = peak response of the corresponding analyte from the *Sample solution*

r_S = peak response of the corresponding analyte from the *Standard solution*

C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (µg/mL)

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

Acceptance criteria: 90.0%–110.0% of the labeled amount of $C_{21}H_{28}O_2$, 90.0%–110.0% of the labeled amount of $C_{20}H_{24}O_2$

PERFORMANCE TESTS

• **Dissolution ⟨711⟩:** Determine the amount of $C_{21}H_{28}O_2$ and $C_{20}H_{24}O_2$ dissolved by employing the following method.

Medium: Polysorbate 80 (5 µg/g) in water; 500 mL

Apparatus 2: 75 rpm

Time: 60 min

Mobile phase: Acetonitrile and water (6:4)

Standard solution: Prepare a solution of USP Levonorgestrel RS and USP Ethinyl Estradiol RS in *Medium* having known concentrations corresponding approximately to the concentrations that would be obtained by dissolving 1 Tablet in 500 mL of *Medium*.

[Note—A volume of alcohol not exceeding 2% of the final total volume of solution may be used to aid in dissolving the Reference Standards.]

Sample solution: Withdraw 15-mL portions of liquid from each vessel, and pass through a polyvinylidene filter, discarding the first 10 mL of the filtrate.

Chromatographic system

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

Mode: LC

Detector: UV 247 nm (for levonorgestrel analysis); a spectrofluorometric detector (for ethinyl estradiol analysis), with an excitation wavelength of 285 nm, and an emission wavelength of 310 nm

Column: 4-mm × 15-cm; packing L7

Flow rate: 1 mL/min

Injection size: 100 µL

System suitability

Sample: *Standard solution*

[Note—The relative retention times for ethinyl estradiol and levonorgestrel are about 0.7 and 1.0, respectively.]

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{21}H_{28}O_2$ and $C_{20}H_{24}O_2$ dissolved:

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

r_U = peak response of the corresponding analyte from the *Sample solution*

r_S = peak response of the corresponding analyte from the *Standard solution*

C_S = concentration of the appropriate USP Reference Standard in the *Standard solution* (µg/mL)

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

Tolerances

Uncoated Tablets: NLT 80% (Q) of the labeled amount of $C_{21}H_{28}O_2$, and 75% (Q) of the labeled amount of $C_{20}H_{24}O_2$ is dissolved.

Coated Tablets: NLT 60% (Q) of the labeled amount of $C_{21}H_{28}O_2$ and $C_{20}H_{24}O_2$ is dissolved.

• **Uniformity of Dosage Units ⟨905⟩:** Meet the requirements

Sample solution: Place 1 Tablet in a 40-mL centrifuge tube, add 10.0 mL of *Mobile phase*, and proceed as directed in the *Assay*.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers.

• **USP Reference Standards ⟨11⟩**

USP Ethinyl Estradiol RS
USP Levonorgestrel RS

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

Topic/Question	Contact	Expert Committee
LEVONORGESTREL AND ETHINYL ESTRADIOL TABLETS	Documentary Standards Support	SM52020 Small Molecules 5
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM52020 Small Molecules 5

Chromatographic Database Information: Chromatographic Database

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