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Esterified Estrogens Tablets

DEFINITION

Esterified Estrogens Tablets contain NLT 90.0% and NMT 115.0% of the labeled amount of esterified estrogens as the total of sodium estrone sulfate and sodium equilin sulfate. The ratio of sodium equilin sulfate to sodium estrone sulfate is NLT 0.071 and NMT 0.20.

IDENTIFICATION

• **A.** The *Sample solution* exhibits peaks for estrone and equilin at relative retention times corresponding to those from the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**

Internal standard solution: 150 µg/mL of 3-*O*-methylestrone in methanol

Standard stock solution: 160 and 70 µg/mL each of USP Estrone RS and USP Equilin RS in alcohol

Standard solution: Pipet 1.0 mL of the *Standard stock solution* and 1.0 mL of the *Internal standard solution* into a suitable centrifuge tube fitted with a tight screw cap or stopper. Evaporate the mixture with the aid of a stream of nitrogen to dryness, maintaining the temperature below 50°. To the dry residue add 15 µL of dried pyridine and 65 µL of bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane. Immediately cover the tube tightly, and allow to stand for 15 min. Add 0.5 mL of toluene.

System suitability stock solution: 2 µg/mL of USP Estradiol RS (17β-estradiol) in alcohol

System suitability solution: Pipet 1.0 mL of *System suitability stock solution*, 1.0 mL of *Standard stock solution*, and 1.0 mL of *Internal standard solution* into a centrifuge tube fitted with a tight screw cap or stopper. Proceed as directed for *Standard solution*, beginning with "Evaporate the mixture…".

Buffer: Mix 79 mL of sodium acetate TS with 21 mL of 1 N acetic acid, and dilute with water to 500 mL. If necessary, adjust the solution with 1 N acetic acid or sodium acetate TS to a pH of 5.2 ± 0.1.

Sample solution: Transfer an equivalent to 2 mg of total esterified estrogens from powdered Tablets (NLT 20), to a 50-mL centrifuge tube, fitted with a polytef-lined screw cap, containing 15 mL of *Buffer* and 1 g of barium chloride. Cap the tube tightly, and shake for 30 min. If necessary, adjust the solution with 1 N acetic acid or sodium acetate to a pH of 5.0 ± 0.5. Sonicate for 30 s, then shake for an additional 30 min. Add a suitable sulfatase enzyme solution equivalent to 2500 Units, and shake for 20 min in a water bath maintained at 50°. Add 15.0 mL of ethylene dichloride to the warm mixture, cap the tube again, and shake by mechanical means for 15 min. Centrifuge for 10 min or until the lower layer is clear. Transfer as much of the organic phase as possible, and dry by passing rapidly through a filter consisting of a pledget of dry glass wool and 5 g of anhydrous sodium sulfate in a small funnel. Protect from loss by evaporation. Transfer 3.0 mL of the solution to a suitable centrifuge tube fitted with a tight screw cap or stopper. Add 1.0 mL of *Internal standard solution*. Proceed as directed under *Standard solution*, beginning with "Evaporate the mixture…".

Chromatographic system

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

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 15-m fused silica capillary column; with a 0.25-µm layer of phase G19

Temperature

Column: 208°

Detector: 260°

Injector port: 260°

Carrier gas: Hydrogen

Flow rate: 2 mL/min

Injection mode: Split

Split flow rate: 40–60 mL/min

Injection size: 1 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—Adjust the operating conditions as necessary to maintain the elution time of the 3-*O*-methylestrone peak between 17 and 25 min.]

[NOTE—The relative retention times for 17β-estradiol, estrone, equilin, and 3-*O*-methylestrone are about 0.29, 0.80, 0.87, and 1.00, respectively.]

Suitability requirements

Resolution: NLT 1.2 between estrone and equilin, *System suitability solution*

Tailing factor: NMT 1.3 for the estrone peak, *System suitability solution*

Relative standard deviation: NMT 2.0% for the estrone peak ratios for NLT 4 injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Separately calculate the percentage of the labeled amount of sodium estrone sulfate and sodium equilin sulfate in the portion of Tablets taken:

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

R_U = ratio of the estrone or equilin peak to the 3-*O*-methylestrone peak area from the *Sample solution*

R_S = ratio of the estrone or equilin peak to the 3-*O*-methylestrone peak area from the *Standard solution*

C_S = concentration of USP Estrone RS or USP Equilin RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of estrone or equilin in the *Sample solution* (µg/mL)

F = factor converting free estrogen to the conjugate sodium salt, 1.377 for estrone and 1.380 for equilin

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS

• **DISINTEGRATION ⟨701⟩**

Simulated intestinal fluid: Dissolve 6.8 g of monobasic potassium phosphate in 250 mL of water, mix, add 190 mL of 0.2 N sodium hydroxide and 400 mL of water. Add 10.0 g of pancreatin, mix, and adjust the resulting solution with 0.2 N sodium hydroxide to a pH of 7.5 ± 0.1. Dilute with water to 1000 mL.

Analysis: Place 1 Tablet in each of the six tubes of the basket, and immerse the basket in water at 25 ± 0.5° for 5 min to remove the coating. Add a disk to each tube, and operate the apparatus using simulated gastric fluid TS, maintained at 37 ± 2°, as the immersion fluid. After 30 min in simulated gastric fluid TS, lift the basket from the fluid, and observe the Tablets.

Acceptance criteria: All the Tablets have disintegrated. If all the Tablets have not disintegrated completely, substitute *Simulated intestinal fluid*, maintained at 37 ± 2°, as the immersion fluid, and continue the test so that the total period of time, including previous exposure to water and simulated gastric fluid TS, does not exceed 90 min.

• **UNIFORMITY OF DOSAGE UNITS ⟨905⟩**

Analysis: Test 10 individual Tablets as directed in the *Assay*, and calculate the average content of esterified estrogens, as the average of the total contents of sodium estrone sulfate and sodium equilin sulfate, in the 10 Tablets.

Acceptance criteria: The requirements are met if the content of each of the Tablets is 85.0%–115.0% of the average content of esterified estrogens. If the content of NMT 2 Tablets falls outside the range of 85.0%–115.0% of the average content but not outside the range of 75.0%–125.0%, test an additional 20 Tablets. The requirements are met if the content of NMT 2 of the 30 Tablets falls outside the limits of 85.0%–115.0% of the average, and no unit is outside the range of 75.0%–125.0% of the average content.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS ⟨11⟩**

USP Equilin RS

USP Estradiol RS

USP Estrone RS

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

Topic/Question	Contact	Expert Committee
ESTERIFIED ESTROGENS TABLETS	Documentary Standards Support	SM52020 Small Molecules 5

Chromatographic Database Information: Chromatographic Database

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