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Esterified Estrogens

DEFINITION

Esterified Estrogens is a mixture of the sodium salts of the sulfate esters of the estrogenic substances, principally estrone. It is a dispersion of the estrogenic substances on a suitable powdered diluent. The content of total esterified estrogens is NLT 90.0% and NMT 110.0% of the labeled amount.

Esterified Estrogens contains NLT 75.0% and NMT 85.0% of sodium estrone sulfate, and NLT 6.0% and NMT 15.0% of sodium equilin sulfate, in such proportion that the total of these two components is NLT 90.0%, of the labeled amount of esterified estrogens.

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 *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 the equivalent to 2 mg of total esterified estrogens from Esterified Estrogens, 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 at between 17 and 25 min.]

[NOTE—The relative retention times for 17β-estradiol, estrone, equilin, and 3-*O*-methylestrone are 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 Esterified Estrogens 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 area to the 3-*O*-methylestrone peak area from the *Sample solution*

R_S = ratio of the estrone or equilin peak area 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 = concentration of the *Sample solution* (µg/mL)

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

Acceptance criteria: 90.0%–110.0% of the labeled amount of total esterified estrogens; 75.0%–85.0% of sodium estrone sulfate; 6.0%–15.0% of sodium equilin sulfate, in such proportion that the total of these two components is NLT 90.0%, of the labeled amount of esterified estrogens

IMPURITIES

• **FREE STEROIDS**

Internal standard solution, Buffer, Standard stock solution, Chromatographic system, and **System suitability solution:** Proceed as directed in the *Assay*.

Free steroids standard solution: Dilute the *Standard stock solution* tenfold. Pipet 1.0 mL of the resulting solution and 1.0 mL of the *Internal standard solution* into a suitable centrifuge tube fitted with a tight screw cap or stopper. Proceed as directed for *Standard solution* in the *Assay*, beginning with "Evaporate the mixture…".

Sample solution: Proceed as directed for *Sample solution* in the *Assay* with the following exceptions: do not add the sulfatase enzyme solution, and transfer 6.0 mL of the filtrate instead of 3.0 mL to the *Sample solution*.

Blank solution: Prepare a reagent blank in the same manner as the *Sample solution*.

System suitability: Proceed as directed in the *Assay* with the additional requirement that the relative standard deviation for the ratio of the peak response of estrone to that of the internal standard in the *Free steroids standard solution* is NMT 5.5%, on the basis of NLT two replicate injections.

Analysis

Samples: *Free steroids standard solution* and *Sample solution*

Calculate the total percentage of estrone and equilin (free stereoids) in the portion of Esterified Estrogens taken:

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

R_U = ratio of the sum of the estrone and equilin peak areas (corrected for any peaks found in the *Blank solution*) to the 3-*O*-methylestrone peak area from the *Sample solution*

R_S = ratio of the estrone peak area to the 3-*O*-methylestrone peak area from the *Free steroids standard solution*

C_S = concentration of USP Estrone RS in the *Free steroids standard solution* (µg/mL)

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

Acceptance criteria: NMT 3.0% of free steroids

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.
- **Labeling:** Label it to state the content of Esterified Estrogens on a weight-to-weight basis.
- **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	Documentary Standards Support	SM52020 Small Molecules 5

Chromatographic Database Information: Chromatographic Database

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