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Conjugated Estrogens Tablets

DEFINITION

Conjugated Estrogens Tablets contain NLT 73.0% and NMT 95.0% of the labeled amount of conjugated estrogens as the total of sodium estrone sulfate and sodium equilin sulfate. The ratio of sodium equilin sulfate to sodium estrone sulfate in the Tablets is NLT 0.35 and NMT 0.65.

IDENTIFICATION

• **A.** The relative retention times of the 17α-dihydroequilin peak, the estrone peak, and the equilin peak from the *Sample solution* correspond to those from the *Standard solution*, as obtained in the *Assay*.

• **B.** The chromatogram of the *Sample solution* in the *Assay* exhibits additional peaks or shoulders, corresponding to 17α-estradiol and 17β-dihydroequilin at retention times of about 0.24 and 0.35, relative to that of 3-*O*-methylestrone.

ASSAY

• **Procedure**

Buffer: Sodium acetate TS, 1 N acetic acid, and water (79:21:400). Adjust with 1 N acetic acid or sodium acetate TS to a pH of 5.2 ± 0.1, if necessary.

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

Standard stock solution: 160, 70, and 50 µg/mL each of USP Estrone RS, USP Equilin RS, and USP 17α-Dihydroequilin RS, respectively, in alcohol

Standard solution: Pipet 1.0 mL of the *Internal standard solution* and 1.0 mL of the *Standard stock 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, mix, and allow to stand for 15 min. Add 0.5 mL of toluene, and mix.

System suitability solution: Pipet 1.0 mL of a 2.0-µg/mL solution of USP Estradiol RS (17β-estradiol) in alcohol, 1.0 mL of *Internal standard solution*, and 1.0 mL of *Standard stock solution* into a centrifuge tube fitted with a tight screw cap or stopper. Proceed as directed for the *Standard solution*, beginning with "Evaporate the mixture…".

Sample solution: If the Tablets are sugar coated, carefully remove the color and sugar coatings with water, leaving the shellac coating intact, and dry under nitrogen. Transfer an equivalent to 2 mg of total conjugated estrogens from NLT 20 finely powdered Tablets, to a 50-mL centrifuge tube fitted with a polytef-lined screw-cap and 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 rapidly passing 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 for the *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; bonded with a 0.25-µm layer of phase G19

Temperatures

Column: 208°

Detector: 260°

Injection port: 260°

Carrier gas: Hydrogen

Flow rate: 2 mL/min

Injection mode: Split

Split flow rate: 40–60 mL/min

Injection volume: 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 17–25 min.]

[NOTE—The relative retention times for 17β-estradiol, 17α-dihydroequilin, estrone, equilin, and 3-*O*-methylestrone are about 0.29, 0.30, 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% of the estrone peak ratios for NLT four injections of the *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

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 = peak response ratio of the appropriate analyte to that of the internal standard from the *Sample solution*

R_S = peak response ratio of the appropriate analyte to that of the internal standard 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 = conversion factor (ratio of molecular weight of sodium salts to free estrogen), 1.381

Acceptance criteria: 73.0%–95.0% of the labeled amount of conjugated estrogens as the total of sodium estrone sulfate and sodium equilin sulfate. The ratio of sodium equilin sulfate to sodium estrone sulfate is NLT 0.35 and NMT 0.65.

PERFORMANCE TESTS

• **DISSOLUTION ⟨711⟩:** Proceed as directed for *Extended-Release* articles.

Test 1 (for products labeled as 0.3-, 0.45-, and 0.625-mg tablets)**:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Times: 2, 5, and 8 h

Buffer: 0.025 M monobasic potassium phosphate

Mobile phase: Acetonitrile and *Buffer* (1:3)

Standard solution: Transfer 10 Tablets to a 1000-mL volumetric flask, dilute with water to volume, and stir vigorously by mechanical means for at least 3 h. Pipet a filtered 100-mL aliquot of the solution into a 900-mL volumetric flask, and dilute with water to volume.

Sample solution: Filter a portion of the solution under test. It is recommended that the filters selected be tested for binding affinity.

Chromatographic system

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

Mode: LC

Detector: UV 205 nm

Column: 4.6-mm × 3.0-cm; 3-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 20–200 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for equilin sulfate and estrone sulfate are about 0.9 and 1.0, respectively. The estrone sulfate peak is the last major peak in the chromatogram. If estrone is present, it may be retained on the column for a period longer than 50 min and interfere in later chromatographic runs.]

Suitability requirements

Resolution: NLT 1.5 between equilin sulfate and estrone sulfate

Relative standard deviation: NMT 1.5% for the estrone sulfate peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of estrone sodium sulfate dissolved:

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

r_U = estrone sulfate peak response from the *Sample solution*

r_S = estrone sulfate peak response from the *Standard solution*

Tolerances: See *Table 1*.

Table 1

Time (h)	Amount Dissolved (%)
2	19–49
5	66–96
8	NLT 80

The percentages of the labeled amount of estrone sodium sulfate dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

Test 2 (for products labeled as 0.9-mg tablets)**:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium, Apparatus, Times, Mobile phase, Standard solution, Sample solution, Chromatographic system, System suitability, and **Analysis:** Proceed as directed for *Test 1*.

Tolerances: See *Table 2*.

Table 2

Time (h)	Amount Dissolved (%)
2	12–37
5	57–85
8	NLT 80

The percentages of the labeled amount of estrone sodium sulfate dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

Test 3 (for products labeled as 1.25- and 2.50-mg tablets)**:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium, Apparatus, Mobile phase, Standard solution, Sample solution, Chromatographic system, System suitability, and **Analysis:** Proceed as directed for *Test 1*.

Times: 2, 5, 8, and 12 h

Tolerances: See *Table 3*.

Table 3

Time (h)	Amount Dissolved (%)
2	3–22

Time (h)	Amount Dissolved (%)
5	37–67
8	66–96
12	NLT 80

The percentages of the labeled amount of estrone sodium sulfate dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

Test 4 (for products labeled as 1.25-mg tablets)**:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

Medium: Acetate buffer, pH 4.5; 900 mL

Apparatus 2: 50 rpm, with sinkers

Times: 2, 4, 8, and 12 h

Buffer: 0.025 M monobasic potassium phosphate

Mobile phase: Acetonitrile and *Buffer* (22:78)

Standard solution: Weigh 20 Tablets and determine the average tablet weight. Grind the Tablets to a uniform fine powder. Weigh a portion of the powdered Tablets equivalent to the average tablet weight, transfer to a 900-mL volumetric flask, and dilute with *Medium* to volume. Stir vigorously by mechanical means for at least 2 h or until the dissolution of the powder is complete. Pass a portion of the extract through a suitable filter of 10-µm pore size.

Sample solution: Pass a portion of the solution under test through a suitable filter of 10-µm pore size. It is recommended that the filters selected be tested for binding affinity.

Chromatographic system

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

Mode: LC

Detector: UV 210 nm

Column: 3.2-mm × 5.0-cm; 5-µm packing L1

Flow rate: 0.8 mL/min

Injection volume: 20–200 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for equilin sulfate and estrone sulfate are about 0.9 and 1.0, respectively. The estrone sulfate peak is the last major peak in the chromatogram. If estrone is present, it may be retained on the column for a period longer than 50 min and interfere in later chromatographic runs.]

Suitability requirements

Resolution: NLT 1.2 between equilin sulfate and estrone sulfate

Relative standard deviation: NMT 2.0% for the estrone sulfate peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of estrone sulfate dissolved:

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

r_U = estrone sulfate peak response from the *Sample solution*

r_S = estrone sulfate peak response from the *Standard solution*

Tolerances: See *Table 4*.

Table 4

Time (h)	Amount Dissolved (%)
2	11–31

Time (h)	Amount Dissolved (%)
4	43–63
8	75–95
12	NLT 87

The percentages of the labeled amount of estrone sodium sulfate dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

Test 5 (for products labeled as 0.3-, 0.45-, and 0.625-mg tablets)**:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.

Medium, Apparatus, Mobile phase, Standard solution, Sample solution, Chromatographic system, System suitability, and **Analysis:** Proceed as directed for *Test 4*.

Times: 1, 3, and 8 h

Tolerances: See *Table 5*.

Table 5

Time (h)	Amount Dissolved (%)
1	6–26
3	48–68
8	NLT 87

The percentages of the labeled amount of estrone sodium sulfate dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

Test 6 (for products labeled as 0.9-mg tablets)**:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.

Medium, Apparatus, Mobile phase, Standard solution, Sample solution, Chromatographic system, System suitability, and **Analysis:** Proceed as directed for *Test 4*.

Times: 1, 3, and 8 h

Tolerances: See *Table 6*.

Table 6

Time (h)	Amount Dissolved (%)
1	3–23
3	41–61
8	NLT 80

The percentages of the labeled amount of estrone sodium sulfate dissolved at the times specified conform to *Acceptance Table 2* in ⟨711⟩.

• **Uniformity of Dosage Units**

Analysis: Assay 10 individual Tablets as directed in the *Assay*, and calculate the average content of conjugated 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 conjugated 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%, assay 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 that 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.
- **Labeling:** The labeling indicates the Tablet strength and states with which in vitro *Dissolution Test* the product complies.
- **USP Reference Standards** ⟨11⟩

USP 17α-Dihydroequilin RS

Estra-1,3,5(10),7-tetraene-3,17α-diol.

$C_{18}H_{22}O_2$ 270.37

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
CONJUGATED ESTROGENS TABLETS	Documentary Standards Support	SM52020 Small Molecules 5

Chromatographic Database Information: Chromatographic Database

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