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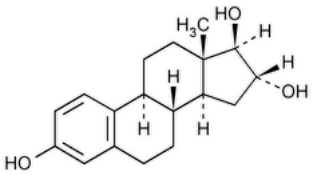
Estriol



$C_{18}H_{24}O_3$ 288.38

Estra-1,3,5(10)-triene-3,16,17-triol, (16α,17β)-;

Estriol CAS RN®: 50-27-1.

DEFINITION

Estriol contains NLT 97.0% and NMT 102.0% of estriol ($C_{18}H_{24}O_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲**Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197K.**▲ (CN 1-May-2020) Meets the requirements

Change to read:

• **B.** ▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2019)

ASSAY

Change to read:

• **Procedure**

▲**Solution A:** Water

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	65	35
3.0	65	35
14.0	56	44
17.0	10	90
17.1	65	35
20.0	65	35

Diluent: Methanol and water (1:1)

System suitability solution: 100 µg/mL of USP Estriol RS and 5 µg/mL of USP Estriol Related Compound A RS in *Diluent*. Sonicate to dissolve as needed.

Standard solution: 100 µg/mL of USP Estriol RS in *Diluent*. Sonicate to dissolve as needed.

Sample solution: 100 µg/mL of Estriol in *Diluent*. Sonicate to dissolve as needed.

Chromatographic system

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

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 2.6-µm packing L1

Column temperature: 32°

Flow rate: 1.0 mL/min

Injection volume: 25 µL

System suitability

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

[NOTE—See *Table 2* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.2 between estriol related compound A and estriol, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of estriol ($C_{18}H_{24}O_3$) in the portion of Estriol taken:

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

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

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

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

▲ (USP 1-May-2019)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

• **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.1%

Change to read:

• **ORGANIC IMPURITIES**

▲**Solution A, Solution B, Diluent,** and **Chromatographic system:** Proceed as directed in the *Assay*.

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0.0	65	35
3.0	65	35
40.0	35	65
41.0	65	35
45.0	65	35

System suitability stock solution A: 200 µg/mL of USP Estriol RS prepared as follows. Transfer a suitable quantity of USP Estriol RS to an appropriate volumetric flask. Add 50% of the total flask volume of methanol to dissolve, and sonicate to fully dissolve the solids. Dilute with water to volume and mix well.

System suitability stock solution B: 100 µg/mL each of USP Estriol Related Compound A RS, USP Estrone RS, and USP Estradiol RS prepared as follows. Transfer a suitable quantity of each Reference Standard to an appropriate volumetric flask. Add 80% of the total flask volume of methanol to dissolve, and sonicate to fully dissolve the solids. Dilute with methanol to volume and mix well.

System suitability solution: 100 µg/mL of USP Estriol RS and 5 µg/mL each of USP Estriol Related Compound A RS, USP Estrone RS, and USP Estradiol RS prepared as follows. Transfer 10-mL of *System suitability stock solution A* into a 20-mL volumetric flask. Add 1 mL of *System suitability stock solution B* and 1 mL of water. Dilute with *Diluent* to volume and mix well. Sonicate to dissolve as needed.

Standard solution: 0.4 µg/mL of USP Estriol RS and 2 µg/mL each of USP Estriol Related Compound A RS, USP Estrone RS, and USP Estradiol RS in *Diluent*. Sonicate to dissolve as needed.

Sample solution: 400 µg/mL of Estriol prepared as follows. Transfer a suitable quantity of Estriol to an appropriate volumetric flask. Add 50% of the total flask volume of methanol to dissolve, and sonicate to fully dissolve the solids. Dilute with water to volume and mix well.

System suitability

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

[NOTE—See *Table 3* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.2 between estriol related compound A and estriol; NLT 1.2 between estrone and estradiol, *System suitability solution*

Relative standard deviation: NMT 10.0%; estriol, estriol related compound A, estrone, and estradiol, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of estriol related compound A, estrone, and estradiol in the portion of Estriol taken:

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

r_U = peak response of estriol related compound A, estrone, or estradiol from the *Sample solution*

r_S = peak response of the corresponding USP Reference Standard from the *Standard solution*

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

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

Calculate the percentage of any individual impurity in the portion of Estriol taken:

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

r_U = peak response of any individual impurity from the *Sample solution*

r_S = peak response of estriol from the *Standard solution*

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

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

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
16β,17α-Estriol[a]	0.94	0.5
Estriol related compound A	0.97	0.5
Estriol	1.00	—
16β-Estriol[b]	1.53	0.5
17α-Estriol[c]	1.60	0.5
Estrone	2.30	0.5
Estradiol	2.34	0.5
3-*O*-Methyl estriol[d]	2.64	0.5
Any individual unspecified impurity	—	0.10
Total impurities[e]	—	1

[a] Estra-1,3,5(10)-triene-3,16β,17α-triol.

[b] Estra-1,3,5(10)-triene-3,16β,17β-triol.

[c] Estra-1,3,5(10)-triene-3,16α,17α-triol.

[d] 3-Methoxy-(16α,17β)-estra-1,3,5(10)-triene-16,17-diol.

[e] Total impurities exclude estriol related compound A.

▲ (USP 1-May-2019)

SPECIFIC TESTS

• **Optical Rotation** ⟨781S⟩, *Procedures, Specific Rotation*

Sample solution: 4 mg/mL, in dioxane

Acceptance criteria: +54° to +62°

• **Loss on Drying** ⟨731⟩

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 0.5% of its weight

Delete the following:

▲• **Completeness of Solution**

Sample solution: 50 mg/mL in pyridine

Acceptance criteria: The solution is clear and free from undissolved solid.▲ (USP 1-May-2019)

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.

Change to read:

• **USP Reference Standards** ⟨11⟩

▲ USP Estradiol RS▲ (USP 1-May-2019)

USP Estriol RS

▲ USP Estriol Related Compound A RS

Estra-1,3,5(10),9(11)-tetraene-3,16α,17β-triol.

$C_{18}H_{22}O_3$ 286.37

USP Estrone RS

▲ (USP 1-May-2019)

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

Topic/Question	Contact	Expert Committee
ESTRIOL	Documentary Standards Support	SM52020 Small Molecules 5

Chromatographic Database Information: Chromatographic Database

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