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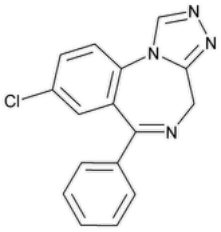
Estazolam



$C_{16}H_{11}ClN_4$ 294.74

4*H*-[1,2,4]Triazolo[4,3-*a*][1,4]benzodiazepine, 8-chloro-6-phenyl-;

8-Chloro-6-phenyl-4*H*-*s*-triazolo[4,3-*a*][1,4]benzodiazepine CAS RN®: 29975-16-4; UNII: 36S3EQV54C.

DEFINITION

Estazolam contains NLT 98.0% and NMT 102.0% of estazolam ($C_{16}H_{11}ClN_4$), calculated on the dried basis.

IDENTIFICATION

Change to read:

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

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**

Buffer: 2.8 g/L of monobasic potassium phosphate in water. Adjust with 1 N sodium hydroxide to a pH of 6.5.

Mobile phase: Acetonitrile, methanol, and *Buffer* (10:35:55)

Standard stock solution: 0.5 mg/mL of USP Estazolam RS in *Mobile phase*

Standard solution: 0.02 mg/mL of USP Estazolam RS in water from *Standard stock solution*

Sample stock solution: 0.5 mg/mL of Estazolam in *Mobile phase*

Sample solution: 0.02 mg/mL of Estazolam in water from *Sample stock solution*

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 3-µm packing L11

Flow rate: 1 mL/min

Injection volume: 25 µL

Run time: 2.5 times the retention time of the estazolam peak

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of estazolam ($C_{16}H_{11}ClN_4$) in the portion of Estazolam 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 Estazolam RS in the *Standard solution* (mg/mL)

C_U = concentration of Estazolam in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- **Residue on Ignition ⟨281⟩:** NMT 0.1%
- **Organic Impurities**

Solution A: Acetonitrile

Solution B: Water

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	40	60
20	90	10
23	90	10
30	40	60
35	40	60

[Note—The gradient was established on an HPLC system with a dwell volume of approximately 1.0 mL.]

System suitability solution: 1 µg/mL each of USP Estazolam RS, USP Nordazepam RS, and USP Estazolam Related Compound A RS in acetonitrile

Standard solution: 1 µg/mL of USP Estazolam RS in acetonitrile

Sample solution: 1 mg/mL of Estazolam in acetonitrile

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 3-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

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

Suitability requirements

Resolution: NLT 2.0 between nordazepam and estazolam related compound A, *System suitability solution*

Tailing factor: NMT 1.2 for estazolam, *Standard solution*

Relative standard deviation: NMT 2.0% for estazolam, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

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

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

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

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

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

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

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Estazolam	1.0	—	—
Nordazepam[a]	1.4	1.3	0.1
Estazolam related compound A[b]	1.6	1.0	0.1
Formamido chlorobenzophenone[c]	2.0	1.5	0.1
Bischloroacetylbenzo phenone[d]	2.2	1.5	0.1
Aminochlorobenzo phenone[e]	2.6	1.4	0.1
Chloroacetamido chlorobenzophenone[f]	2.7	1.2	0.1
Any individual, unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.5

[a] 7-Chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one.

[b] 5-Chloro-2-(3-chloromethyl-4*H*-1,2,4-triazol-4-yl)-benzophenone.

[c] *N*-(2-Benzoyl-4-chlorophenyl) formamide.

[d] 2-(*N*-Chloroacetyl-*N*-chloroacetylhydrazonomethyl)amino-5-chlorobenzophenone.

[e] (2-Amino-5-chlorophenyl) phenyl-methanone.

[f] *N*-(2-Benzoyl-4-chlorophenyl)-2-chloroacetamide.

SPECIFIC TESTS

• **Loss on Drying ⟨731⟩**

Analysis: Dry at 105° for 4 h.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

• **USP Reference Standards ⟨11⟩**

USP Estazolam RS

4*H*-[1,2,4]Triazolo[4,3-*a*][1,4]benzodiazepine, 8-chloro-6-phenyl-;
8-Chloro-6-phenyl-4*H*-*s*-triazolo[4,3-*a*][1,4]benzodiazepine.
$C_{16}H_{11}ClN_4$ 294.74

USP Estazolam Related Compound A RS

5-Chloro-2-(3-chloromethyl-4*H*-1,2,4-triazol-4-yl)-benzophenone.
$C_{16}H_{11}Cl_2N_3O$ 332.18

USP Nordazepam RS

7-Chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one.
$C_{15}H_{11}ClN_2O$ 270.71

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

Topic/Question	Contact	Expert Committee
ESTAZOLAM	Documentary Standards Support	SM42020 Small Molecules 4

Chromatographic Database Information: Chromatographic Database

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