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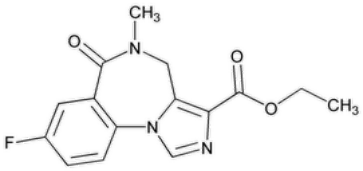
Flumazenil



$C_{15}H_{14}FN_3O_3$ 303.29

4*H*-Imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylic acid, 8-fluoro-5,6-dihydro-5-methyl-6-oxo-, ethyl ester;

Ethyl 8-fluoro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate CAS RN®: 78755-81-4; UNII: 40P7XK9392.

DEFINITION

Flumazenil contains NLT 98.0% and NMT 102.0% of flumazenil ($C_{15}H_{14}FN_3O_3$), 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**

Solution A: 800 mL of water, adjusted with phosphoric acid to a pH of 2.0 ± 0.05

Mobile phase: Methanol, tetrahydrofuran, and *Solution A* (13:7:80)

System suitability solution: 6.4 µg/mL each of USP Flumazenil RS and USP Flumazenil Related Compound B RS in *Mobile phase*

Standard solution: 1.0 mg/mL of USP Flumazenil RS in *Mobile phase*

Sample solution: 1.0 mg/mL of Flumazenil in *Mobile phase*

Chromatographic system

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

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 5 µL

Run time: NLT 1.3 times the retention time of flumazenil

System suitability

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

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

Suitability requirements

Resolution: NLT 4.0 between flumazenil related compound B and flumazenil, *System suitability solution*

Tailing factor: NMT 1.5 for flumazenil, *System suitability solution*

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

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of flumazenil ($C_{15}H_{14}FN_3O_3$) in the portion of Flumazenil 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 Flumazenil RS in the *Standard solution* (mg/mL)

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

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

IMPURITIES

• **Residue on Ignition ⟨281⟩:** NMT 0.1%

Change to read:

• **Limit of Flumazenil Related Compound C**

Diluent: Alcohol and chloroform (50:50)

Standard solution A: 0.5 mg/mL of USP Flumazenil RS and 0.6 µL/mL of USP Flumazenil Related Compound C RS in *Diluent*

Standard solution B: 0.1 mg/mL of USP Flumazenil RS and ▲0.12 µL/mL▲ (ERR 1-Jun-2018) of USP Flumazenil Related Compound C RS from *Standard solution A* in *Diluent*

Sample solution: 50 mg/mL of Flumazenil in *Diluent*

Chromatographic system

(See *Chromatography ⟨621⟩, Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: Chloroform, glacial acetic acid, alcohol, and water (75:15:7.5:2.5)

Spray reagent: Dissolve 0.5 g of ninhydrin in 90 mL of alcohol, and add 10 mL of glacial acetic acid.

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Proceed as directed in the chapter. Dry the plate for 10 min in a current of cold air, and view under short-wavelength UV light. Spray the plate with *Spray reagent*, and heat at 105° for 15 min. The R_F values of the analytes with their corresponding methods of detection are listed in *Table 1*.

Table 1

Compound	R_F	Detection
Flumazenil	About 0.8	UV
Flumazenil related compound C	About 0.04	Ninhydrin

Acceptance criteria: Any spot at an R_F value corresponding to flumazenil related compound C of the *Sample solution* is not more intense than the corresponding spot of *Standard solution B* (NMT 0.2%).

• **Organic Impurities**

Solution A, Mobile phase, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay* except use a *Run time* of NLT 3 times the retention time of flumazenil.

Standard solution: 1 µg/mL of USP Flumazenil RS in *Mobile phase*

Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of each impurity in the portion of Flumazenil taken:

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

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Flumazenil RS in the *Standard solution* (mg/mL)

C_U = concentration of Flumazenil in the *Sample solution* (mg/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 (%)
Flumazenil related compound A[a]	0.4	1.1	0.2
Flumazenil dione[b]	0.5	1.5	0.2
Desfluoro flumazenil[c]	0.7	1.3	0.2
Flumazenil related compound B	0.8	1.1	0.2
Flumazenil	1.0	—	—
Chloroflumazenil[d]	2.2	1.1	0.2
Any individual unknown impurity	—	1.0	0.1
Total impurities	—	—	0.5

[a] 8-Fluoro-5-methyl-6-oxo-5,6-dihydro-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylic acid; also known as 8-Fluoro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazol-[1,5-*a*][1,4]benzodiazepine-3-carboxylic acid.

[b] 7-Fluoro-4-methyl-3,4-dihydro-1*H*-benzo[*e*][1,4]diazepine-2,5-dione.

[c] Ethyl 5-methyl-6-oxo-5,6-dihydro-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylate.

[d] Ethyl 8-chloro-5-methyl-6-oxo-5,6-dihydro-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylic acid.

SPECIFIC TESTS

- **Bacterial Endotoxins Test ⟨85⟩:** NMT 25.0 USP Endotoxin Units/mg of flumazenil
- **Loss on Drying ⟨731⟩**

  Analysis: Dry at 105° for 3 h.

  Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.
- **USP Reference Standards ⟨11⟩**

USP Flumazenil RS

USP Flumazenil Related Compound B RS

Ethyl 8-hydroxy-5-methyl-6-oxo-5,6-dihydro-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylate;
also known as Ethyl 8-hydroxy-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazol-[1,5-*a*][1,4]benzodiazepine-3-carboxylate.

$C_{15}H_{15}N_3O_4$ 301.30

USP Flumazenil Related Compound C RS

1,1-Diethyoxy-*N*,*N*-dimethylmethanamine;
also known as *N*,*N*-Dimethylformamide diethyl acetal.

$C_7H_{17}NO_2$ 147.22

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

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

Chromatographic Database Information: Chromatographic Database

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