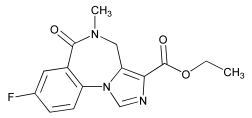
Flumazenil



$C_{15}H_{14}FN_3O_3$ 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 [*78755-81-4*].

» Flumazenil contains not less than 98.0 percent and not more than 102.0 percent of $C_{15}H_{14}FN_3O_3$, calculated on the dried basis.

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. USP Flumazenil Related Compound C RS.*

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: The retention time of the major peak for flumazenil in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Melting range, *Class Ia* ⟨741⟩**:** between 198° and 202°.

Bacterial endotoxins ⟨85⟩**—**It contains not more than 25.0 USP Endotoxin Units per mg of flumazenil.

Loss on drying ⟨731⟩**—**Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩**:** not more than 0.1%.

Heavy metals, *Method II* ⟨231⟩**:** 0.002%.

Related compounds—

TEST 1—

Ninhydrin solution—Dissolve 0.5 g of ninhydrin in 90 mL of alcohol, and add 10 mL of glacial acetic acid.

Diluent—Prepare a mixture of alcohol and chloroform (1 : 1).

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture (see *Chromatography* ⟨621⟩).

Test solution—Transfer about 250 mg of Flumazenil, accurately weighed, to a 5-mL volumetric flask. Dissolve in and dilute with *Diluent* to volume, and mix.

Standard solution 1—Prepare a solution of USP Flumazenil RS and USP Flumazenil Related Compound C RS in *Diluent* having known concentrations of about 0.5 mg per mL and about 0.6 µL per mL, respectively.

Standard solution 2—Dilute 2.0 mL of *Standard solution 1* with *Diluent* to 10.0 mL.

Application volume: 10 µL.

Developing solvent system: a mixture of chloroform, glacial acetic acid, alcohol, and water (75 : 15 : 7.5 : 2.5).

Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* ⟨621⟩. Dry the plate for 10 minutes in a current of cold air, and view under short-wavelength UV light. Spray the plate with *Ninhydrin solution,* and heat at 105° for 15 minutes. The R_F values of analytes are as follows.

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

Any spot at an R_F value corresponding to flumazenil related compound C in the chromatogram obtained from the *Test solution* is not more intense than the corresponding spot in the chromatogram obtained from *Standard solution 2:* not more than 0.2% is found.

TEST 2—

Diluted phosphoric acid, pH 2.0, Mobile phase, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay*.

Standard solution—Dilute the *Standard preparation* quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 1 µg per mL.

Test solution—Use the *Assay preparation.*

Procedure—Separately inject equal volumes (about 5 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms for at least three times the retention time of the flumazenil peak, and measure the areas for the major peaks. Calculate the percentage of any impurity in the portion of Flumazenil taken by the formula:

$$100(C_S / C_U)(r_i / r_S)(1/F)$$

in which C_S and C_U are the concentrations, in mg per mL, of flumazenil in the *Standard solution* and the *Test solution,* respectively; r_i is the peak area for any impurity in the *Test solution;* r_S is the peak area for flumazenil in the *Standard solution;* and F is the relative response factor for each of the known impurities relative to flumazenil. [NOTE—F values are given for all the impurities, along with the corresponding limits, in the *Table* below.]

Organic volatile impurities, *Method IV* ⟨467⟩**:** meets the requirements.

Solvent—Use dimethyl sulfoxide.

(Official until July 1, 2008)

Assay—

Diluted phosphoric acid, pH 2.0—Adjust 800 mL of water with phosphoric acid to a pH of 2.0 ± 0.05.

Mobile phase—Prepare a filtered and degassed mixture of *Diluted phosphoric acid, pH 2.0,* methanol, and tetrahydrofuran (80 : 13 : 7). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Dissolve appropriate quantities of USP Flumazenil RS and USP Flumazenil Related Compound B RS in *Mobile phase,* and dilute, stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 6.4 µg per mL of each compound.

Standard preparation—Dissolve an accurately weighed quantity of USP Flumazenil RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 1.0 mg per mL of flumazenil.

Assay preparation—Transfer about 25.0 mg of Flumazenil, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph 5 µL of the *System suitability solution,* and record the peak responses: the relative retention times are about 0.8 for flumazenil related compound B and 1.0 for flumazenil; the resolution, *R,* between flumazenil related compound B and flumazenil is not less than 4.0; the column efficiency is not less than 1500 theoretical plates for the flumazenil peak; and the tailing factor is not more than 1.5 for the flumazenil peak. Chromatograph 5 µL of the *Standard preparation,* and record the peak responses: the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the flumazenil peaks. Calculate the percentage of $C_{15}H_{14}FN_3O_3$ in the portion of Flumazenil taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which C_S and C_U are the concentrations, in mg per mL, of flumazenil in the *Standard preparation* and the *Assay preparation,* respectively; and r_U and r_S are the peak areas obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Compound Name	Relative Retention Time	Relative Response Factor	Limit (%)
Flumazenil related compound A	about 0.4	1.1	0.2
7-Fluoro-4-methyl-3,4-dihydro-2,5*H*-1,4-benzodiazepine-2,5-dione	about 0.5	1.5	0.2
Ethyl 5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo-[1,5-*a*][1,4]benzodiazepine-3-carboxylate	about 0.7	1.3	0.2
Flumazenil related compound B	about 0.8	1.1	0.2
Flumazenil	1.0	—	—
Ethyl 8-chloro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo-[1,5-*a*][1,4]benzodiazepine-3-carboxylate	about 2.2	1.1	0.2
Any individual unknown impurity	—	1.0	0.1
Total	—	—	0.5