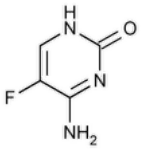
Status: Currently Official on 14-Feb-2025
Official Date: Official as of 01-May-2022
Document Type: USP Monographs
DocId: GUID-80257960-5270-4CB4-8A8A-48716A0B1B7C_5_en-US
DOI: https://doi.org/10.31003/USPNF_M33280_05_01
DOI Ref: qsv2v

Flucytosine



$C_4H_4FN_3O$ 129.09

Cytosine, 5-fluoro-;

5-Fluorocytosine CAS RN®: 2022-85-7; UNII: D83282DT06.

Change to read:

DEFINITION

Flucytosine contains ▲NLT 98.0% and NMT 102.0%▲ (USP 1-May-2022) of flucytosine ($C_4H_4FN_3O$), calculated on the dried basis.

IDENTIFICATION

• **A. Spectroscopic Identification Tests ⟨197⟩, *Ultraviolet-Visible Spectroscopy*:** 197U

Analytical wavelength: 285 nm

Medium: Dilute hydrochloric acid in water (1 in 100).

Sample solution: 8 µg/mL of flucytosine in *Medium*

Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by NMT 2.0%.

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-2022)

ASSAY

Change to read:

• **Procedure**

▲**Buffer:** 13.6 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.0.

Solution A: *Buffer* and methanol (98:2)

Solution B: *Buffer* and methanol (85:15)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
10	0	100
35	0	100
35.1	100	0
45	100	0

Diluent: Dissolve 13.6 g of monobasic potassium phosphate in 980 mL of water. Add 20 mL of methanol and mix.

Standard solution: 0.05 mg/mL of USP Flucytosine RS in *Diluent*

Sample solution: 0.05 mg/mL of Flucytosine in *Diluent*

Chromatographic system

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

Mode: LC

Detector: UV 284 nm

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

Flow rate: 1.1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of flucytosine ($C_4H_4FN_3O$) in the portion of Flucytosine taken:

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

r_U = peak response of flucytosine from the *Sample solution*

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

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

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

Acceptance criteria: 98.0%–102.0% on the dried basis▲ (USP 1-May-2022)

OTHER COMPONENTS

Delete the following:

▲• **Fluorouracil**

Diluent: Glacial acetic acid and water (4:1)

Standard solution: 0.025 mg/mL solution of USP Fluorouracil RS in *Diluent*

Sample solution: 25 mg/mL flucytosine in *Diluent*

Application volume: 20 µL

Chromatographic system

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

Mode: TLC

Adsorbent: 0.5-mm layer of chromatographic silica gel mixture

Developing solvent system: Chloroform and glacial acetic acid (13:7)

Analysis

Samples: *Standard solution* and *Sample solution*

Locate the spots on the plate by observing under short-wavelength UV radiation.

Acceptance criteria: Any spot from the *Sample solution* is not greater in size and intensity than the spot at the respective R_F produced by the *Standard solution*, corresponding to NMT 0.1% of fluorouracil.▲ (USP 1-May-2022)

IMPURITIES

Add the following:

▲• **Organic Impurities**

Buffer, Solution A, Solution B, Mobile phase, and **Diluent:** Prepare as directed in the *Assay*.

Sensitivity solution: 0.1 µg/mL of USP Flucytosine RS in *Diluent*

Standard solution: 0.00030 mg/mL of USP Fluorouracil RS, 0.00045 mg/mL of USP Fluorouracil Related Compound F RS, and 0.00015 mg/mL of USP Flucytosine RS in *Diluent*

Sample solution: 0.3 mg/mL of Flucytosine in *Diluent*

Chromatographic system

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

Mode: LC

Detector: UV 260 nm

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

Flow rate: 1.1 mL/min

Injection volume: 20 µL

System suitability

Samples: *Sensitivity solution* and *Standard solution*

Suitability requirements

Relative standard deviation: NMT 5% each for flucytosine, fluorouracil, and fluorouracil related compound F, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of fluorouracil in the portion of Flucytosine taken:

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

r_U = peak response of fluorouracil from the *Sample solution*

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

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

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

Calculate the percentage of fluorouracil related compound F in the portion of Flucytosine taken:

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

r_U = peak response of fluorouracil related compound F from the *Sample solution*

r_S = peak response of fluorouracil related compound F from the *Standard solution*

C_S = concentration of USP Fluorouracil Related Compound F RS in the *Standard solution* (mg/mL)

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

Calculate the percentage of any individual impurity in the portion of Flucytosine 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 flucytosine from the *Standard solution*

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

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

Acceptance criteria: See *Table 2*. The reporting threshold is 0.03%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Flucytosine	1.0	—
Fluorouracil	2.0	0.1
Fluorouracil related compound F	7.9	0.15
Any individual unspecified impurity	—	0.05
Total impurities	—	0.3▲ (USP 1-May-2022)

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

Change to read:

• **▲Limit of Fluoride Ion▲** (USP 1-May-2022)

[Note—All glassware and/or plasticware used in this test should be scrupulously clean and even free from trace amounts of fluoride. The use of plasticware to contain the solutions while the potential is measured is recommended.]

Buffer: Transfer 110 g of sodium chloride into a 2-L volumetric flask, add 1 g of sodium citrate and 700 mL of water, and dissolve with shaking. Carefully add 150 g of sodium hydroxide, and dissolve with shaking. Cool to room temperature, and while stirring, cautiously add

450 mL of glacial acetic acid. Cool to room temperature, add 600 mL of isopropyl alcohol, dilute with water to volume, and mix. The pH of this solution is between 5.0 and 5.5.

Standard stock solution: 1 mg/mL of fluoride ion solution in water, prepared as follows. Transfer 2.211 g of sodium fluoride, previously dried at 150° for 4 h, into a 1-L volumetric flask, and dissolve in 200 mL of water. Add 1.0 mL of sodium hydroxide solution in water (1 in 250), dilute with water to volume, and mix. Store the solution in a closed plastic container.

Standard solutions: 1, 3, 5, and 10 µg/mL of fluoride from *Standard stock solution* in *Buffer*

Sample solution: 10 mg/mL of Flucytosine in *Buffer*

Analysis

Samples: *Standard solutions* and *Sample solution*

Concomitantly measure the potential (see *Titrimetry* ⟨541⟩), in mV, of the *Standard solutions* and the *Sample solution*, with a suitable pH meter equipped with a fluoride-specific ion electrode and a glass-sleeved calomel reference electrode that has been modified in the following manner. Mix 70 mL of freshly prepared saturated potassium chloride solution with 30 mL of isopropyl alcohol, fill the electrode with the clear supernatant, and allow the electrode to remain in the mixture for NLT 2 h before use, or preferably overnight. When taking the measurements, transfer the solution to a 150-mL beaker, and immerse the electrodes. Insert a polytef-coated stirring bar into the beaker, place the beaker on a magnetic stirrer having an insulated top, and allow to stir until equilibrium is attained (1–2 min). Rinse and dry the electrodes between measurements, taking care not to scratch the crystal in the specific ion electrode. Measure the potential of each *Standard solution*, and plot the fluoride concentration, in mg/100 mL, versus the potential, in mV, on semilogarithmic paper. Measure the potential of the *Sample solution*, and determine from the standard curve the fluoride concentration, in mg/100 mL.

Calculate the percentage of fluoride in the portion of Flucytosine taken:

$$\text{Result} = C/10$$

C = concentration of fluoride from the standard curve (mg/100 mL)

Acceptance criteria: NMT 0.05% of fluoride

SPECIFIC TESTS

• **Loss on Drying** ⟨731⟩

Analysis: Dry at 105° for 4 h.

Acceptance criteria: NMT 1.5%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers.

Change to read:

• **USP Reference Standards** ⟨11⟩

USP Flucytosine RS

USP Fluorouracil RS

▲ USP Fluorouracil Related Compound F RS

2-Ethoxy-5-fluoropyrimidin-4(1*H*)-one.

$C_6H_7FN_2O_2$ 158.13▲ (USP 1-May-2022)

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

Topic/Question	Contact	Expert Committee
FLUCYTOSINE	Documentary Standards Support	SM12020 Small Molecules 1

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. 46(5)

Current DocID: GUID-80257960-5270-4CB4-8A8A-48716A0B1B7C_5_en-US

DOI: https://doi.org/10.31003/USPNF_M33280_05_01

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