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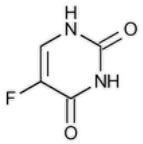
Fluorouracil



$C_4H_3FN_2O_2$ 130.08

2,4(1*H*,3*H*)-Pyrimidinedione, 5-fluoro-;

5-Fluorouracil CAS RN®: 51-21-8; UNII: U3P01618RT.

DEFINITION

Fluorouracil contains NLT 98.0% and NMT 102.0% of fluorouracil ($C_4H_3FN_2O_2$), calculated on the dried basis.

[**Caution—**Great care should be taken to prevent inhaling particles of Fluorouracil and exposing the skin to it.]

IDENTIFICATION

Change to read:

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

Change to read:

• **B.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Medium: pH 4.7 acetate buffer prepared from 8.4 g of sodium acetate and 3.35 mL of glacial acetic acid mixed with water to make 1000 mL

Sample solution: 10 µg/mL in *Medium*

Acceptance criteria: Meets the requirements

• **C.** 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: 6.8 g/L of monobasic potassium phosphate in water. Adjust with 5 M potassium hydroxide to a pH of 5.7 ± 0.1.

Mobile phase: Acetonitrile and *Buffer* (5:95)

Standard solution: 10 µg/mL of USP Fluorouracil RS in water

Sample solution: 10 µg/mL of Fluorouracil in water

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

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

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

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

Tailing factor: NMT 1.5, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of fluorouracil ($C_4H_3FN_2O_2$) in the portion of Fluorouracil 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*

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

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

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

IMPURITIES

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

• **Organic Impurities**

Protect the *Standard solution* and *Sample solution* from light.

Mobile phase: 6.8 g/L of monobasic potassium phosphate in water. Adjust with 5 M potassium hydroxide to a pH of 5.7 ± 0.1.

Standard solution: 0.1 µg/mL each of USP Fluorouracil RS, USP Fluorouracil Related Compound A RS, USP Fluorouracil Related Compound B RS, and USP Uracil RS, and 0.2 µg/mL of USP Fluorouracil Related Compound E RS in *Mobile phase*

Sample solution: 0.1 mg/mL of Fluorouracil in *Mobile phase*

Chromatographic system

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

Mode: LC

Detector: UV 266 nm

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

Flow rate: 1.0 mL/min

Injection volume: 20 µL

Run time: NLT 3 times the retention time of the fluorouracil peak

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2 between the uracil and fluorouracil peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of fluorouracil related compound A, fluorouracil related compound B, and uracil in the portion of Fluorouracil taken:

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

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

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

C_S = concentration of relevant fluorouracil related compound in the *Standard solution* (mg/mL)

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

Calculate the percentage of 5-methoxyuracil, fluorouracil related compound E, and any unspecified impurity in the portion of Fluorouracil taken:

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

r_U = peak response of each impurity 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 Fluorouracil in the *Sample solution* (mg/mL)

F = relative response factor for each individual impurity (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard any impurity peaks less than 0.05%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fluorouracil related compound A[a]	0.5	—	0.15

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Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fluorouracil related compound B[b]	0.7	—	0.15
Uracil	0.9	—	0.15
Fluorouracil	1.0	—	—
5-Methoxyuracil[c]	1.6	0.67	0.15
Fluorouracil related compound E[d]	1.9	0.77	0.15
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.5

[a] Pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione.

[b] Dihydropyrimidine-2,4,5(3*H*)-trione.

[c] 5-Methoxypyrimidine-2,4(1*H*,3*H*)-dione.

[d] 5-Chloropyrimidine-2,4(1*H*,3*H*)-dione.

• **Limits of Fluorouracil Related Compound F and Urea**

Diluent: Methanol and water (1:1)

Standard solution A: 0.025 mg/mL of USP Fluorouracil Related Compound F RS in *Diluent*

Standard solution B: 0.02 mg/mL of USP Urea RS in methanol

Sample solution: 10 mg/mL of Fluorouracil in *Diluent*

Chromatographic system

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

Mode: TLC

Adsorbent: Chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: Ethyl acetate, methanol, and water (70:15:15)

Reagent: Prepare a 10-mg/mL solution of *p*-dimethylaminobenzaldehyde in anhydrous alcohol. Prepare a mixture of this solution and hydrochloric acid (10:1).

Analysis

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

Procedure: Develop with *Developing solvent system*, followed by air drying. Examine the plate under UV light at 254 nm for fluorouracil related compound F. Spray the plate at least twice with *Reagent*, and dry the plate in an oven at 80° for 3–4 min. Examine the plate under daylight for urea. [Note—The urea produces a yellow spot, and fluorouracil is not detected by the spray.]

Acceptance criteria: The spot of fluorouracil related compound F in the *Sample solution* is not more intense than the spot of fluorouracil related compound F from *Standard solution A* (NMT 0.25%). The spot of urea in the *Sample solution* is not more intense than the spot of urea from *Standard solution B* (NMT 0.2%).

SPECIFIC TESTS

• **Loss on Drying ⟨731⟩**

Analysis: Dry under vacuum over phosphorus pentoxide at 80° for 4 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

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

• **USP Reference Standards ⟨11⟩**

USP Fluorouracil RS

USP Fluorouracil Related Compound A RS

Pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione.

$C_4H_4N_2O_3$ 128.09

USP Fluorouracil Related Compound B RS

Dihydropyrimidine-2,4,5(3*H*)-trione.

$C_4H_4N_2O_3$ 128.09

USP Fluorouracil Related Compound E RS

5-Chloropyrimidine-2,4(1*H*,3*H*)-dione.

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$C_4H_3ClN_2O_2$ 146.53

USP Fluorouracil Related Compound F RS

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

$C_6H_7FN_2O_2$ 158.13

USP Uracil RS

Uracil.

$C_4H_4N_2O_2$ 112.09

USP Urea RS

Urea.

CH_4N_2O 60.06

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

Topic/Question	Contact	Expert Committee
FLUOROURACIL	Documentary Standards Support	SM32020 Small Molecules 3

Chromatographic Database Information: Chromatographic Database

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