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Fluorouracil Injection

DEFINITION

Fluorouracil Injection is a sterile solution of Fluorouracil in Water for Injection, prepared with the aid of Sodium Hydroxide. It contains NLT 90.0% and NMT 110.0% of the labeled amount of fluorouracil ($C_4H_3FN_2O_2$).

[NOTE—If a precipitate is formed as a result of exposure to low temperatures, redissolve it by heating to 60° with vigorous shaking, and allow to cool to body temperature prior to use.]

IDENTIFICATION

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

Change to read:

• **B. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** ▲197A or▲ (USP 1-Aug-2024) 197M

Sample for 197M: Carefully acidify a portion of Injection, equivalent to 100 mg of fluorouracil, with glacial acetic acid. Stir, and slightly chill the solution to precipitate the fluorouracil. Collect the precipitate, wash with 1 mL of water, and then dry under vacuum over phosphorus pentoxide at 80° for 4 h.

Acceptance criteria: Meets the requirements

Add the following:

▲• **C.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Aug-2024)

ASSAY

Change to read:

• **Procedure**

Buffer: 6.8 g/L of potassium phosphate, monobasic 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: Nominally 10 µg/mL of fluorouracil in water, from Injection

Chromatographic system

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

Mode: LC

Detector: UV 254 nm. ▲For *Identification C*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-Aug-2024)

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%

Tailing factor: NMT 1.5

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of fluorouracil ($C_4H_3FN_2O_2$) in the portion of Injection taken:

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

r_U = peak response ▲of fluorouracil▲ (USP 1-Aug-2024) from the *Sample solution*

r_S = peak response ▲of fluorouracil▲ (USP 1-Aug-2024) from the *Standard solution*

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

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

Acceptance criteria: 90.0%–110.0%

Add the following:

▲IMPURITIES

• **Organic Impurities**

Mobile phase: 0.77 g/L of ammonium acetate in water. Adjust with formic acid to a pH of 5.60–5.65.

System suitability solution: 0.2 mg/mL of USP Fluorouracil RS and 1.0 µg/mL each of USP Fluorouracil Related Compound A RS, USP Fluorouracil Related Compound B RS, USP Uracil RS, and USP Fluorouracil Related Compound E RS in water

Sensitivity solution: 0.1 µg/mL of USP Fluorouracil Related Compound E RS in water

Standard solution: 0.3 µg/mL each of USP Fluorouracil RS, USP Fluorouracil Related Compound A RS, USP Fluorouracil Related Compound B RS, USP Uracil RS, and USP Fluorouracil Related Compound E RS in water

Sample solution: Nominally 0.2 mg/mL of fluorouracil in water, from Injection

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 4-µm packing L11

Flow rate: 1.0 mL/min

Injection volume: 10 µL

Run time: NLT 3 times the retention time of fluorouracil

System suitability

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

Suitability requirements

Resolution: NLT 2.0 between fluorouracil related compound A and fluorouracil related compound B; NLT 2.0 between fluorouracil related compound B and uracil; NLT 2.0 between uracil and fluorouracil; NLT 2.0 between fluorouracil and fluorouracil related compound E, *System suitability solution*

Relative standard deviation: NMT 5.0% for each component, *Standard solution*

Signal-to-noise ratio: NLT 10 for flurouracil related compound E, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

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

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

r_U = peak area of each specified impurity from the *Sample solution*

r_S = peak area of each specified impurity from the *Standard solution*

C_S = concentration of corresponding Reference Standard in the *Standard solution* (mg/mL)

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

Calculate the percentage of any unspecified impurity in the portion of Injection taken:

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

r_U = peak area of each unspecified impurity from the *Sample solution*

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

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

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

Acceptance criteria: See *Table 1*. The reporting threshold is 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Fluorouracil related compound A	0.50	0.2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Fluorouracil related compound B	0.71	0.2
Uracil	0.86	0.2
Fluorouracil	1.0	—
Fluorouracil related compound E	1.71	0.2
Any unspecified impurity	—	0.2
Total impurities[a]	—	1.0

[a] Total impurities does not include urea, which is tested separately.

• **Limit of Urea**

Mobile phase: 12.7 g/L of sodium nitrate with 0.1% (v/v) of triethylamine in water

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

Sample solution: Nominally 25 mg/mL of fluorouracil in *Mobile phase*, from Injection

Chromatographic system

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

Mode: LC

Detector: Refractive index

Column: 7.8-mm × 30-cm; 6-µm packing L39

Temperatures

Detector: 45°

Column: 35°

Flow rate: 0.8 mL/min

Injection volume: 50 µL

Run time: NLT 1.5 times the retention time of urea

Attenuation: 250,000 nano Refractive Index Units

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0%

Tailing factor: NMT 2.0

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of urea in the portion of Injection taken:

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

r_U = peak area of urea from the *Sample solution*

r_S = peak area of urea from the *Standard solution*

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

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

Acceptance criteria: NMT 3.0%▲ (USP 1-Aug-2024)

SPECIFIC TESTS

Change to read:

• **Bacterial Endotoxins Test ⟨85⟩:** ▲Meets the requirements▲ (USP 1-Aug-2024)

Add the following:

▲• **Particulate Matter in Injections ⟨788⟩:** Meets the requirements▲ (USP 1-Aug-2024)

• **pH ⟨791⟩:** 8.6–9.4

Add the following:

▲• **Sterility Test ⟨71⟩:** Meets the requirements▲ (USP 1-Aug-2024)

• **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products ⟨1⟩*.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in single-dose containers, preferably of Type I glass, and store at controlled room temperature. Avoid freezing and exposure to light.

Change to read:

• **Labeling:** Label it to indicate the expiration date ▲▲ (USP 1-Aug-2024) .

Change to read:

• **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.

$C_4H_3ClN_2O_2$ 146.53

USP Uracil RS

Uracil.

$C_4H_4N_2O_2$ 112.09

USP Urea RS

Urea.

CH_4N_2O 60.06▲ (USP 1-Aug-2024)

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

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

Chromatographic Database Information: Chromatographic Database

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