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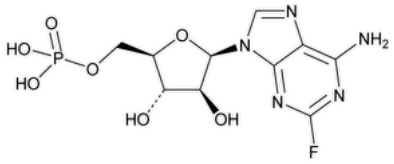
Fludarabine Phosphate



$C_{10}H_{13}FN_5O_7P$ 365.21

9*H*-Purin-6-amine, 2-fluoro-9-(5-*O*-phosphono-β-D-arabinofuranosyl)-;

9-β-D-Arabinofuranosyl-2-fluoroadenine 5'-(dihydrogen phosphate) CAS RN®: 75607-67-9; UNII: 1X9VK9O1SC.

DEFINITION

Fludarabine Phosphate contains NLT 98.0% and NMT 102.0% of fludarabine phosphate ($C_{10}H_{13}FN_5O_7P$), calculated on the anhydrous, solvent-free basis.

[CAUTION—Fludarabine phosphate is potentially cytotoxic. Great care should be taken to prevent inhaling particles and exposing the skin to it.]

IDENTIFICATION

Change to read:

• **A.** ▲**SPECTROSCOPIC IDENTIFICATION TESTS ⟨197⟩, *Infrared Spectroscopy*:** 197A or 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: 10 mM monobasic potassium phosphate

Mobile phase: Methanol and *Solution A* (6:94)

Standard solution: 0.02 mg/mL of USP Fludarabine Phosphate RS in *Mobile phase*

Sample solution: 0.02 mg/mL of Fludarabine Phosphate in *Mobile phase*

Chromatographic system

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

Mode: LC

Detector: UV 260 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of fludarabine phosphate ($C_{10}H_{13}FN_5O_7P$) in the portion of Fludarabine Phosphate 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 Fludarabine Phosphate RS in the *Standard solution* (mg/mL)

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

Acceptance criteria: 98.0%–102.0% on the anhydrous, solvent-free basis

IMPURITIES

- **Chloride**

Standard stock solution: 82.4 µg/mL of sodium chloride in water

Standard solution: Transfer 2.0 mL of *Standard stock solution* to a test tube, add 13.0 mL of water, and mix.

Sample solution: Transfer 50.0 mg of Fludarabine Phosphate to a test tube, add 15 mL of water to dissolve, and heat gently if necessary.

Analysis: Add 1.0 mL of nitric acid to the *Standard solution* and *Sample solution*, and place each in separate, colorless test tubes containing 1.0 mL of silver nitrate TS.

Acceptance criteria: NMT 0.2%; the *Sample solution* shows less turbidity than the *Standard solution*.

- **Limit of Free Phosphate**

Reagent solution: Mix 4 g of finely powdered ammonium molybdate and 0.1 g of finely powdered ammonium vanadate in a 150-mL beaker. Add 70 mL of water, and grind the particles using a glass rod. A clear solution is obtained within a few minutes. Add 20 mL of nitric acid, adjust to room temperature, and dilute with water to 100 mL.

Standard solution: 7.16 µg/mL of potassium dihydrogen phosphate in water. Transfer 2.0 mL of this solution to a test tube.

Sample solution: Transfer 10 mg of Fludarabine Phosphate in 2.0 mL of water to a test tube and heat gently.

Blank: 2.0 mL of water in a test tube

Analysis: To each of the test tubes containing the *Standard solution*, *Sample solution*, and *Blank*, add 2.0 mL of *Reagent solution*.

Acceptance criteria: NMT 0.1%; the color of the *Standard solution* must be more intense than that of the *Blank*. Viewed downward in diffuse daylight against a white background, the yellow coloration of the *Sample solution* must not be more intense than that of the *Standard solution*.

- **Limit of Sodium**

Standard stock solution: 2.54 mg/mL of sodium chloride in water. Sodium chloride is previously dried at 105° for 2 h.

Standard solution: 1 µg/mL of sodium in water, from *Standard stock solution*

Sample solution: 0.5 mg/mL of Fludarabine Phosphate in water

Instrumental conditions

Mode: Flame photometry

Analytical wavelength: Sodium emission line at 589.0 nm

Blank: Water

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria: NMT 0.2%; the emission response of the *Sample solution* is NMT that of the *Standard solution*.

- **Organic Impurities, Procedure 1: Early-Eluting Impurities**

Mobile phase, Standard solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability solution: 10 mg of Fludarabine Phosphate in 10 mL of 0.1 N hydrochloric acid. Heat the solution at 80° in a water bath for 15 min.

Sensitivity solution: 0.5 µg/mL of USP Fludarabine Phosphate RS in *Mobile phase*, from the *Standard solution*

Sample solution: 1 mg/mL of Fludarabine Phosphate in *Mobile phase*

System suitability

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

Suitability requirements

Resolution: NLT 2.0 between the iso-ara-guanine monophosphate and isoguanine peaks, *System suitability solution*

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

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

Analysis

Sample: *Sample solution*

Calculate the percentage of each early-eluting impurity in the portion of Fludarabine Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (1/F_1) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of fludarabine phosphate from the *Sample solution*

F_1 = relative response factor for each individual impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Iso-ara-guanine-monophosphate[a]	0.26	0.25	0.8
Isoguanine[b]	0.34	0.40	0.2
3',5'-Diphosphate analog[c]	0.42	0.53	0.4
Any individual, unspecified impurity	<1.0	1.0	0.1
Fludarabine phosphate	1.0	—	—

[a] 6-Amino-9-β-D-arabinofuranosyl-2-oxo-1*H*-purine 5′-(dihydrogen phosphate).

[b] 6-Amino-1*H*-purin-2(9*H*)-one.

[c] 9-β-D-Arabinofuranosyl-2-fluoroadenine 3′,5′-bis(dihydrogen phosphate).

• **Organic Impurities, Procedure 2: Late-Eluting Impurities**

Solvent A: 10 mM monobasic potassium phosphate

Mobile phase: Methanol and *Solvent A* (1:4)

Standard solution and **Chromatographic system:** Proceed as directed in the *Assay*.

Sensitivity solution and **Sample solution:** Prepare as directed in *Organic Impurities, Procedure 1: Early-Eluting Impurities*.

System suitability

Samples: *Standard solution* and *Sensitivity solution*

Suitability requirements

Tailing factor: NMT 2.0, *Standard solution*

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

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

Analysis

Sample: *Sample solution*

Calculate the percentage of each late-eluting impurity in the portion of Fludarabine Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (1/F_2) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of fludarabine phosphate from the *Sample solution*

F_2 = relative response factor for each individual impurity (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fludarabine phosphate	1.0	—	—
2-Fluoroadenine[a]	1.5	2.0	0.1
2-Fluoro-ara-adenine[b]	1.9	1.7	0.2
2-Ethoxyphosphate analog[c]	2.5	0.56	0.2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any individual, unspecified impurity	>1.0	1.0	0.1
Total unspecified impurities[d]	—	—	0.5
Total impurities[e]	—	—	1.5

[a] 2-Fluoro-9*H*-purin-6-amine.

[b] 9-β-D-Arabinofuranosyl-2-fluoroadenine.

[c] 2-Ethoxy-9-β-D-arabinofuranosyladenine 5′-(dihydrogen phosphate).

[d] The sum of all unspecified impurities found in *Organic Impurities, Procedure 1: Early-Eluting Impurities* and *Organic Impurities, Procedure 2: Late-Eluting Impurities*.

[e] The sum of all impurities found in *Organic Impurities, Procedure 1: Early-Eluting Impurities* and *Organic Impurities, Procedure 2: Late-Eluting Impurities*.

• **Limit of Alcohol**

Standard solution: 0.50 mg/mL of alcohol in dimethylformamide

Sample solution: 50 mg/mL of Fludarabine Phosphate in dimethylformamide

Blank: Dimethylformamide

Chromatographic system

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

Mode: GC equipped with a headspace injector

Detector: Flame ionization

Column: 0.25-mm × 30-m; 1.4-µm coating of phase G43

Temperatures

Injection port: 160°

Detector: 250°

Column: See *Table 3*.

Table 3

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	10
40	5	70	—
70	30	220	—

Carrier gas: Helium

Flow rate: 27 cm/s

Sample

Volume: 2 mL/vial. [Note—Seal the vials using a flanged cap so that the cap can no longer be turned.]

Conditioning temperature: 80°

Conditioning time: 60 min

Injection volume: 1.0 mL

System suitability

Samples: *Standard solution* and *Blank*

[Note—The retention time for alcohol is about 3 min.]

Suitability requirements

Relative standard deviation: NMT 4.0% for three injections, *Standard solution*

Peak interference: No peak at the retention time for alcohol, *Blank*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of alcohol in the portion of Fludarabine Phosphate taken:

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

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

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

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

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

[NOTE—Use the percentage obtained to calculate the *Assay* result on the solvent-free basis.]

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS ⟨61⟩** and **TESTS FOR SPECIFIED MICROORGANISMS ⟨62⟩:** The total aerobic microbial count is NMT 10^3 cfu/g.
- **OPTICAL ROTATION ⟨781S⟩**, *Procedures, Specific Rotation*

  Sample solution: 5 mg/mL in water

  Acceptance criteria: +10° to +14°
- **WATER DETERMINATION ⟨921⟩**, *Method I*: NMT 3.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store in a refrigerator.
- **USP REFERENCE STANDARDS ⟨11⟩**

  USP Fludarabine Phosphate RS

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

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

Chromatographic Database Information: Chromatographic Database

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