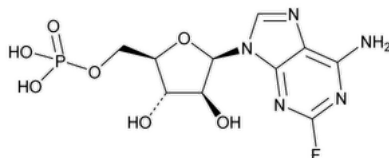


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Fludarabine Phosphate



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

9H-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: 1X9VK901SC.

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