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Fludarabine Phosphate Injection

DEFINITION

Fludarabine Phosphate Injection is a sterile solution of Fludarabine Phosphate in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of fludarabine phosphate ($C_{10}H_{13}FN_5O_7P$).

[**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⟩, *Ultraviolet-Visible Spectroscopy*: 197U**▲ (CN 1-May-2020)

  Solution: 27 µg/mL in 0.1 M hydrochloric acid

- **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: 6.9 g/L of monobasic sodium phosphate monohydrate in water (50 mM). Adjust with 1.0 N sodium hydroxide to a pH of 4.5 ± 0.2.

  Mobile phase: Methanol and *Solution A* (3:47)

  Standard solution: 0.1 mg/mL of USP Fludarabine Phosphate RS in *Solution A*

  Sample solution: Equivalent to 0.1 mg/mL of fludarabine phosphate from Injection diluted with *Solution A*

  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 mL/min

  Injection size: 20 µL

  System suitability

  Sample: *Standard solution*

  Suitability requirements

  Tailing factor: NMT 1.8

  Relative standard deviation: NMT 1%

  Analysis

  Samples: *Standard solution* and *Sample solution*

  Calculate the percentage of the labeled amount of Fludarabine Phosphate ($C_{10}H_{13}FN_5O_7P$) in the portion of Injection 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 = nominal concentration of fludarabine phosphate in the *Sample solution* (mg/mL)

  Acceptance criteria: 95.0%–105.0%

IMPURITIES

Organic Impurities

- **Procedure 1: Early-Eluting Impurities (Impurities Eluting Before Fludarabine)**

  Solution A: 10 mM monobasic potassium phosphate in water

  Mobile phase: *Solution A* and methanol (47:3)

  System suitability solution: 1 mg/mL of fludarabine phosphate in 0.1 N hydrochloric acid. Heat the solution at 80° in a water bath for 15 min.

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

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

Sample solution: Equivalent to 1 mg/mL of fludarabine phosphate from Injection diluted with *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 mL/min

Injection size: 10 µL

System suitability

Samples: *Standard solution*, *System suitability solution*, and *Quantitative limit 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, *Quantitative limit solution*

Analysis

Samples: *Standard solution* and *Sample solution*

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

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

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

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

F = relative response factor (see *Impurity Table 1*)

Acceptance criteria See *Impurity Table 1.*

Impurity Table 1

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

[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). It is a process impurity and controlled in the drug substance monograph.

• **Procedure 2: Late-Eluting Impurities (Impurities Eluting After Fludarabine)**

Solution A, Standard solution, Quantitative limit solution, Sample solution, and **Chromatographic system:** Proceed as directed in *Procedure 1:Early-Eluting Impurities*

Mobile phase: *Solution A* and methanol (4:1)

System suitability

Samples: *Standard solution* and *Quantitative limit solution*

Suitability requirements

Tailing factor: NMT 2.0, *Standard solution*

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

Signal-to-noise ratio: NLT 10, *Quantitative limit solution*

Analysis

Samples: *Standard solution* and *Sample solution*

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

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

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

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

F = relative response factor (see *Impurity Table 2*)

Acceptance criteria

Individual impurities: See *Impurity Table 2*.

Total impurities: The sum of all fludarabine phosphate degradation products found in *Procedure 1* and *Procedure 2* is NMT 2.0%.

Impurity Table 2

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

[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). It is a process impurity and controlled in the drug substance monograph.

SPECIFIC TESTS

- **Bacterial Endotoxins Test ⟨85⟩:** NMT 7.7 USP Endotoxin Units/mg of fludarabine phosphate
- **Sterility Tests ⟨71⟩:** Meets the requirements when tested as directed under *Test for Sterility of the Product to be Examined*, *Membrane Filtration*
- **pH ⟨791⟩:** 6.0–7.1
- **Particulate Matter in Injections ⟨788⟩:** Meets the requirements
- **Injections and Implanted Drug Products ⟨1⟩:** Meets the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, preferably of Type I glass, protected from light. 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 INJECTION	Documentary Standards Support	SM32020 Small Molecules 3

Chromatographic Database Information: Chromatographic Database

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