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Fludarabine Phosphate for Injection

DEFINITION

Fludarabine Phosphate for Injection 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)

Sample solution: 27 µg/mL in 0.1 M hydrochloric acid

Acceptance criteria: Meets the requirements

• **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 of 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 stock solution: 1 mg/mL of fludarabine phosphate in *Mobile phase* prepared as follows. Inject 2.0 mL of *Mobile phase* into each of five vials of Fludarabine Phosphate for Injection. Transfer the contents of the vials into a 250-mL volumetric flask, using *Mobile phase* rinses. Dilute with *Mobile phase* to volume.

Sample solution: 0.02 mg/mL of fludarabine phosphate in *Mobile phase*, from *Sample stock solution*

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 2.0%

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 Fludarabine Phosphate for 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%

PERFORMANCE TESTS

• **Uniformity of Dosage Units ⟨905⟩:** Meets the requirements

IMPURITIES

• **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: Use *Sample stock solution* as directed in the *Assay*.

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 for Injection 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	0.26	0.25	1.0
Isoguanine	0.34	0.40	0.2
3',5'-Diphosphate analog[a]	0.42	—	—
Any other individual degradation product	<1.0	1.0	0.2
Fludarabine phosphate	1.0	—	—

[a] This is a process impurity and controlled in the drug substance monograph.

• **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 for Injection 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	1.5	2.0	0.2
2-Fluoro-ara-adenine	1.9	1.7	0.2
2-Ethoxyphosphate analog[a]	2.5	—	—
Any other individual degradation product	>1.0	1.0	0.2
Total impurities[b]	—	—	2.0

[a] This is a process impurity and controlled in the drug substance monograph.

[b] The sum of all degradation products found in *Organic Impurities Procedure 1* and *Organic Impurities Procedure 2*.

SPECIFIC TESTS

- **Bacterial Endotoxins Test ⟨85⟩:** NMT 7.7 USP Endotoxin Units/mg of fludarabine phosphate
- **pH ⟨791⟩:** 7.2–8.2
- **Sterility Tests ⟨71⟩:** Meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined*, *Membrane Filtration*
- **Water Determination, *Method I* ⟨921⟩:** NMT 5.0%
- **Constituted Solution:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products ⟨1⟩, Specific Tests, Completeness and clarity of solutions*.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve as described in *Packaging and Storage Requirements ⟨659⟩, Injection Packaging, Packaging for constitution*, between 2° and 30°, or at controlled room temperature.
- **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 FOR INJECTION	Documentary Standards Support	SM32020 Small Molecules 3

Chromatographic Database Information: Chromatographic Database

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