BRIEFING

Azacitidine for Injection. A new USP Pending Monograph is proposed based on validated methods of analysis. The liquid chromatographic procedure used in the *Assay* is based on analyses performed with the Inertsil ODS-3V brand of L1 column. The typical retention time for azacitidine is about 7.1 min. The liquid chromatographic procedure used in the test for *Organic Impurities* is based on analyses performed with the Orosil C18 brand of L1 column. The typical retention time for azacitidine is about 19.7 min.

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Add the following:

▸Azacitidine for Injection

Draft 1

DEFINITION

Azacitidine for Injection is a sterile lyophilized powder of Azacitidine. It contains an amount of Azacitidine equivalent to NLT 95.0% and NMT 110.0% of the labeled amount of azacitidine ($C_8H_{12}N_4O_5$).

[**CAUTION—**Azacitidine is a potent cytotoxic agent. Great care should be taken to prevent inhaling particles and exposing the skin to it.]

IDENTIFICATION

- **A. ULTRAVIOLET ABSORPTION** ⟨**197U**⟩
  Sample solution: 10 µg/mL of Azacitidine in water
  Acceptance criteria: Exhibits a characteristic wavelength maxima at 240 nm
- **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**
  Store the *Standard solution* and *Sample solution* at 2°–8°.
  Buffer: 1 mL/L of triethylamine in water. Adjust with dilute phosphoric acid to a pH of 6.8.
  Mobile phase: Methanol and *Buffer* (5:95)
  Diluent: 10.0 g/L of sodium bisulfite in water. Adjust with dilute sulfuric acid to a pH of 2.5.
  Standard solution: 1 mg/mL of USP Azacitidine RS in *Diluent*
  Sample solution: Equivalent to 1.0 mg/mL of azacitidine in *Diluent* from Azacitidine for Injection prepared as follows. Reconstitute a suitable number of vials (NLT 2) with an appropriate amount of *Diluent*, based on the labeled amount of azacitidine.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 270 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Flow rate: 1 mL/min
  Injection volume: 10 µL
  Autosampler temperature: 5°
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Relative standard deviation: NMT 2.0%
  Tailing factor: NMT 2.0
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of azacitidine ($C_8H_{12}N_4O_5$) in the portion of Azacitidine 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 Azacitidine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of azacitidine in the *Sample solution* (mg/mL)

  Acceptance criteria: 95.0%–110.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS,** *Weight Variation* ⟨**905**⟩**:** Meets the requirements

IMPURITIES

- **ORGANIC IMPURITIES**
  Store the *Standard solution* and *Sample solution* at 2°–8°.
  Solution A: 1.54 g/L of ammonium acetate in water
  Solution B: Acetonitrile, methanol, and *Solution A* (20:30:50)
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
8	100	0
20	85	15
25	85	15
30	70	30
40	50	50
45	100	0
55	100	0

  Diluent: 10.0 g/L of sodium bisulfite in water. Adjust with dilute sulfuric acid to a pH of 2.5.
  System suitability solution: 2 mg/mL of USP Azacitidine RS in *Diluent*
  Standard solution: 0.01 mg/mL of USP Azacitidine RS in *Diluent* from *System suitability solution*
  Sample solution: Equivalent to 2 mg/mL of azacitidine in *Diluent*, prepared by reconstituting the vial with an appropriate amount of *Diluent*, based on the labeled amount of azacitidine
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 210 nm
  Column: 4.6-mm × 25-cm; 3-µm packing L1
  Flow rate: 0.8 mL/min
  Injection volume: 5 µL
  Refrigerated autosampler temperature: 5°
  System suitability
  Samples: *System suitability solution* and *Standard solution*
  Suitability requirements
  Tailing factor: NMT 2.0, *System suitability solution*
  Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Azacitidine for Injection taken:

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

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

r_S = peak response of USP Azacitidine RS from the *Standard solution*

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

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

Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.04%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Azacitidine related compound C (isomer-1),[a] (isomer-2),[b] (isomer-3),[c] and (isomer-4)[d]	0.32, 0.33, 0.46, and 0.50	1.2 (Sum of related compound C isomers)
Formyl amidine analog[e]	0.62	2.5
Any unspecified impurity	—	0.20
Azacitidine	1.0	—
Total impurities[f]	—	3.0

[a] 1-β-D-Ribofuranosyl-3-guanylurea.
[b] *N*-(Diaminoethylene)*N*′-(β-D-ribofuranosyl)carbamimidic acid.
[c] 1-β-D-Ribofuranosyl-3-aminocarbonyl guanidine.
[d] 1-β-D-Ribofuranosyl-3-iminohydroxymethyl guanidine.
[e] *N*-(Formyl amidino)-*N*′-β-D-ribofuranosylurea.
[f] Excluding *N*-(formyl amidino)-*N*′-β-D-ribofuranosylurea.

SPECIFIC TESTS

- **Bacterial Endotoxins ⟨85⟩:** It contains NMT 1.0 USP Endotoxin Units/mg of azacitidine.
- **Sterility Tests ⟨71⟩:** Meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*
- **Water Determination,** *Method I* **⟨921⟩:** NMT 1.0%
- **Particulate Matter in Injections ⟨788⟩:** Meets the requirements for small-volume injections
- **Osmolality and Osmolarity ⟨785⟩**

  Sample solution: Reconstitute the contents of a vial with an appropriate amount of *Diluent*, based on the labeled amount of azacitidine, to obtain 10 mg/mL of azacitidine. Mix 1.4 mL of this solution with 10 mL of 0.9% sodium chloride solution.

  Analysis: Measure the osmolarity of 0.9% sodium chloride solution and *Sample solution*.

  Calculate the osmolarity ratio of the sample against 0.9% sodium chloride solution:

$$\text{Osmolarity ratio} = O_U/O_S$$

  O_U = osmolarity of the *Sample solution*

  O_S = osmolarity of 0.9% sodium chloride solution

  Acceptance criteria: 0.80–1.20
- **Other Requirements:** Meets the requirements in *Injections* ⟨1⟩

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve as described in *Injections* ⟨1⟩, *Containers for Sterile Solids*. Store at controlled room temperature.
- **Labeling:** Label it to indicate that the product is in a sterile form for reconstitution as a suspension for subcutaneous injection or reconstitution as a solution with further dilution for intravenous infusion.
- **USP Reference Standards ⟨11⟩**

  USP Azacitidine RS

  USP Endotoxin RS◄ (1-Jan-2014)