Azacitidine for Injection

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Manufacturers, regulatory authorities, health authorities, researchers, and other stakeholders are invited to provide their feedback and comments on this draft proposal. Manufacturers are also invited to submit samples of their products to the IPC to ensure that the proposed monograph adequately controls the quality of the product(s) they manufacture. Comments and samples received after the last date will not be considered by the IPC before finalizing the monograph.

Please send any comments you may have on this draft document to lab.ipc@gov.in, with a copy to Dr. Gaurav Pratap Singh (email: gpsingh.ipc@gov.in) before the last date for comments.

Document History and Schedule for the Adoption Process

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Further follow-up action as required.	

Azacitidine for Injection

Azacitidine for injection is a sterile lyophilized powder of Azacitidine.

Azacitidine for injection contains not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of azacitidine, $C_8H_{12}N_4O_5$.

The injection is reconstituted by dissolving the contents of the sealed container in requisite amount of *sterile water for injection*. The reconstituted solution should be used immediately after preparation but in any case within the period as recommended by the manufacturer.

Commented [SR1]: Inserted as per comment received from stakeholder

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

NOTE – Azacitidine is a potent cytotoxic agent. Great care should be taken to prevent inhaling particles and exposing the skin to it.

Usual strengths. 100 mg per vial.

Identification

A. A 0.001 per cent w/v of solution of azacitidine in *water* shows absorption maxima at 240 nm.

B. In the assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

Test

Related substances. Determined by liquid chromatography (2.4.14).

Note- Store the test solution and reference solution at 2-8˚.

Solvent mixture. 1.0 per cent w/v solution of *sodium bisulphite* in *water*, adjusted to pH 2.5 with *dilute sulphuric acid*.

Test solution. Reconstitute a vial with an appropriate amount of the solvent mixture, based on the labelled amount of azacitidine to obtain a solution containing 0.2 per cent w/v of azacitidine.

Reference solution (a). A 0.2 per cent w/v solution of *azacitidine IPRS* in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 20.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- sample temperature: 5°,
- mobile phase: A. a buffer solution prepared by dissolving 1.54 g of *ammonium acetate* in 1000 ml of *water*,
  B. a mixture of 20 volumes of *acetonitrile*, 30 volumes of *methanol* and 50 volumes of mobile phase A,
- A gradient programme using the condition given below,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 5 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
8	100	0
20	85	15
25	85	15
30	70	30

40	50	50
45	100	0
55	100	0

Name	Relative retention time
Azacitidine related compound C (isomer-1[1], isomer-2[2], isomer-3[3] and isomer-4[4])	0.32, 0.33, 0.46 and 0.50
Formyl amidine analog[5]	0.62
Azacitidine	1.0

[1] 1-β-$_D$-Ribofuranosyl-3-guanylurea,
[2] *N*-(Diaminoethylene)*N'*-(β-$_D$-ribofuranosyl)carbamimidic acid,
[3] 1-β-$_D$-Ribofuranosyl-3-aminocarbonyl guanidine,
[4] 1-β-$_D$-Ribofuranosyl-3-iminohydroxymethyl guanidine,
[5] *N*-(formyl amidino)-*N'*- β-$_D$-ribofuranosylurea.

Inject reference solution (a) and reference solution (b). The test is not valid unless the tailing factor is not more than 2.0 in the chromatogram obtained with reference solution (a) and the relative standard deviation for replicate injections is not more than 10.0 per cent in the chromatogram obtained with reference solution (b).

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the sum of areas of the peaks corresponding to isomer-1, isomer-2, isomer-3 and isomer-4 (azacitidine related compound C) is not more than 2.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.2 per cent), the area of any peak corresponding to formyl amidine analog is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent), the area of any other secondary peak is not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and the sum of areas of all the secondary peaks other than formyl amidine analog is not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent). Ignore any peak with an area less than 0.08 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.04 per cent).

Water (2.3.43). Not more than 1.0 per cent.

Sterility (2.2.11). Complies with the test for sterility.

Bacterial endotoxins (2.2.3). Not more than 1.0 Endotoxin Units per mg of azacitidine.

Osmolality and Osmolarity (2.4.23). Not less than 0.8 and not more than 1.20

Reconstitute a vial with an appropriate amount of the ~~solvent *mixture*~~*water for injection*, based on the labelled amount of azacitidine to obtain a solution containing 1.0 per cent w/v of azacitidine. Dilute 1.4 ml of the solution to 10 ml with 0.9 per cent w/v sodium chloride solution. Measure the osmolarity of 0.9 per cent w/v sodium chloride solution and the test solution.

Calculate the osmolarity ratio of the test solution against 0.9 per cent w/v solution of sodium chloride.

Osmolarity ratio = O_U/O_S

Where, O_U = Osmolarity of the test solution

O_S = Osmolarity of 0.9 per cent w/v solution of sodium chloride.

Other tests. Comply with the tests stated under Parenteral Preparations (Powder for Injections).

Assay. Determined by liquid chromatography (2.4.14).

Note- Store the test solution and the reference solution at 2-8˚.

Solvent mixture. 1.0 per cent w/v solution of *sodium bisulphite* in *water*, adjusted to pH 2.5 with *dilute sulphuric acid*.

Test solution. Reconstitute a suitable number of vials (not less than 2) with an appropriate amount of the solvent mixture, based on the labelled amount of azacitidine. Pool the content of 2 vials and prepare a composite sample. Dilute a suitable volume of pooled sample with the solvent mixture to obtain a solution having 0.1 per cent w/v of azacitidine.

Reference solution. A 0.1 per cent w/v solution of *azacitidine IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- sample temperature: 5°,
- mobile phase: a mixture of 5 volumes of *methanol*, 95 volumes of 0.1 per cent v/v of *triethylamine* in *water*, adjusted to pH 6.8 with *dilute orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_8H_{12}N_4O_5$ in the injection.

Storage. Store at a temperature, not exceeding ~~30°~~25°.

Labelling. ~~When it is intended for use in preparing injectable dosage forms, T~~the label states that it is in a sterile form for reconstitution ~~or must be subjected to further processing during the preparation of injectable dosage forms~~ as a suspension for subcutaneous injection or reconstitution as a solution with further dilution ~~dilute~~ for intravenous infusion.