Azacitidine for Injection

Azacitidine for Injection is a sterile lyophilized powder consisting of Azacitidine, with or without auxilliary substances. It is filled in sealed containers.

The injection is constituted by dissolving the contents of a sealed container in the requisite amount of Water for Injections immediately before use.

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

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

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, $C_8H_{12}N_4O_5$.

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

Usual strength. 100 mg per vial.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. A 0.001 per cent w/v solution of azacitidine in *water* shows absorption maxima at 240 nm (2.4.7).

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

Tests

Related substances. Determine by liquid chromatography (2.4.14).

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

Solvent mixture. A 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 the solvent mixture and dilute with the solvent mixture 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 0.154 per cent w/v solution of *ammonium acetate* in *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 conditions 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
50	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 the 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 the 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, determined on 0.1 g.

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 the solvent mixture and dilute 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 solution of *sodium chloride*. Measure the osmolarity of 0.9 per cent w/v solution of sodium chloride 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.

Assay. Determine by liquid chromatography (2.4.14).

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

Solvent mixture. A 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 the solvent mixture. Pool the content to prepare a composite sample. Dilute a suitable volume of the 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˚.

Labelling. The label states that it is sterile for reconstitution as a suspension for subcutaneous injection or reconstitution as a solution with further dilution for intravenous infusion.