ANALYTICAL METHOD VALIDATION COMMITTEE FOR NON PHARMACOPOEIAL PRODUCT
DEPARTMENT OF DRUG ADMINISTRATION
National Medicines Laboratory

Sofosbuvir Tablet

Analytical Profile No.: Sof 073/074/AP 010

Sofosbuvir Tablets contain not less than 90.0 % and not more than 110.0 % of the stated amount of Sofosbuvir.

1. Identification:

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

2. Dissolution: Determine by liquid chromatography

2.1 Dissolution test parameters

Apparatus: Paddle

Medium: 900ml 0.05 M Phosphate Buffer pH 6.8. 6.8 gm/L potassium dihydrogen orthophosphate and adjust the pH to 6.8 with sodium hydroxide.

Speed and Time: 75 rpm and 30 minutes

Withdraw a suitable volume of the medium and filter

2.2 Test Solution:

Place 1 tablet in each dissolution vessel and run the apparatus as per above condition and collect the sample solution from each jar at specified time. After the completion of the dissolution, dilute 10 ml of the filtrate to 20 ml with acetonitrile and filter through 0.2 micron filter paper.

2.3 Reference Solution:

Weigh accurately about 25 mg of working standard of sofosbuvir and transfer into 50 ml volumetric flask. Dissolve in the dissolution medium and make up the volume to 50 ml with dissolution medium. Dilute 10 ml of the resulting solution to 20 ml with Acetonitrile. Filter through 0.2 micron filter paper.

2.4 Procedure:

Use chromatographic system as described in the Assay using injection volume 20 µl.

Inject 20 µl of reference solution five/six times. The test is not valid unless the column efficiency is not less than 2000 theoretical plates. The tailing factor is not more than 2.0 and the relative standard deviation for replicate injections in not more than 2.0%.

Inject the reference solution and test solution.

Calculate the release of the drug in each tablet.

2.5 Limit:

D. Not less than 75 % of the stated amount.

3. Assay: Determine by liquid chromatography

3.1 Solvent mixture: Prepare a mixture of Water and Acetonitrile (70:30)

3.2 Test Solution: Weigh individually 20 tablets and crush the tablet to fine powder. Weigh accurately the powder equivalent to 50 mg of sofosbuvir into 100 ml volumetric flask. Add about 70 ml of solvent mixture, sonicate for about 10 minutes and cool the solution to room temperature and make up the volume to 100 ml with solvent mixture. Centrifuge the solution. Dilute 10 ml of the solution to 25 ml with solvent mixture. Filter the solution with 0.2 micron filter paper.

3.3 Reference Solution: Weigh accurately about 25 mg of working standard of sofosbuvir and transfer into 50 ml volumetric flask. Dissolve in the solvent mixture and make up the volume to

50 ml with solvent mixture. Dilute 10 ml of the resulting solution to 25 ml with solvent mixture. Filter through 0.2 micron filter paper.

3.4 Chromatographic Condition:

Column:	C18 (150 x 4.6 mm)
Flow rate:	1.5 ml/min
Wavelength:	263 nm
Injection Volume:	10 µl
Column Temperature:	30 ˚C
Detector:	UV

Mobile Phase:

Mobile phase A: Buffer:Acetonitrile (90:10)

Mobile phase B: Acetonitrile:IPA (80:20)

Mix Mobile phase A : Mobile phase B (80:20). Cool to room temperature and filter the solution through 0.2 micron filter paper using vacuum pump.

Buffer: Weigh accurately about 3.4 g potassium dihydrogen orthophosphate and 4.68 g Octane sulphonic acid salt and transfer in 1000 ml beaker. Add 500 ml of water and sonicate to dissolve. Dilute with water to 1000 ml and adjust the pH to 3.0 with orthophosphoric acid.

3.5 Procedure:

Inject 10 µl of reference solution five/six times. The test is not valid unless the column efficiency is not less than 2000 theoretical plates. The tailing factor is not more than 2.0 and the relative standard deviation for replicate injections in not more than 2.0%.

Inject the reference solution and test solution.

Calculate the content of sofosbuvir in the tablet.

4. Other tests: As per pharmacopoeial requirement