DEPARTMENT OF DRUG ADMINISTRATION
National Medicines Laboratory
ANALYTICAL METHOD VALIDATION COMMITTEE

Sofosbuvir Tablets

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

Sofosbuvir Tablets contain not less than 90 % and not more than 110 % 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.

Tests

2. Dissolution: *Determine by liquid chromatography*

2.1 Dissolution parameter:

Apparatus:	Paddle
Medium:	900 ml 0.05 M Phosphate Buffer pH 6.8
Speed and Time:	75 rpm and 30 minutes
Temperature:	37˚C ± 0.5˚C

Withdraw a suitable volume of the medium and filter

2.2 Test Solution: Dilute 10 ml of the filtrate to 20 ml with acetonitrile and filter through 0.2 µm membrane filter paper.

2.3 Reference Solution: Weigh accurately about 25 mg Sofosbuvir reference standard and transfer into 50 ml volumetric flask, dissolve in dissolution medium and make up the volume with same. Dilute 10 ml of the resulting solution to 20 ml with acetonitrile. Filter through 0.2 µm membrane filter paper.

2.4 Chromatographic System and procedure: Proceed as directed under the Assay using injection volume 20 µl.

2.5 Limit: Not less than 75 % (D) of the stated amount.

3. Assay: *Determine by Liquid Chromatography*

Diluent: Prepare a mixture of water and acetonitrile in the ratio 70:30

3.1 Test Solution: Weigh individually 20 tablets and crush them into fine powder. Weigh accurately the powder equivalent to 50 mg of Sofosbuvir into 100 ml volumetric flask. Add about 70 ml of diluent, sonicate for about 10 minutes, cool the solution to room temperature and make up the volume to 100 ml with diluents. Centrifuge the solution, dilute 10 ml of supernatant solution to 25 ml with diluent. Filter the solution through 0.2 µm membrane filter paper.

3.2 Reference Solution: Weigh accurately about 25 mg Sofosbuvir reference standard and transfer into 50 ml volumetric flask. Dissolve with diluent and make up the volume to 50 ml with same. Dilute 10 ml of the resulting solution to 25 ml with diluent. Filter the solution through 0.2 µm membrane filter paper.

3.3 Chromatographic Condition:

Column: C18 (150 x 4.6 mm)

Injection Volume: 10 µl

Flow rate: 1.5 ml/min

Detector: Spectrophotometer set at: 263 nm

Column Temperature: 30 ˚c

Mobile Phase: a mixture of 80 volumes of solution A and 20 volumes of solution B.

Solution A: Buffer: Acetonitrile (90:10)

Solution B: Acetonitrile: IPA (80:20)

Buffer: Weigh accurately about 3.4 g potassium dihydrogen orthophosphate and 4.68 g Octane sulphonic acid sodium 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.

3.4 Procedure: Inject 10 µl of reference solution five times as per above mentioned chromatographic conditions. 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 10 µl of the test solution and blank solution and calculate the content of Sofosbuvir in each tablet.

4. Other tests: As per pharmacopoeial requirements.