ANALYTICAL METHOD VALIDATION COMMITTEE FOR NON PHARMACOPOEIAL PRODUCT

DEPARTMENT OF DRUG ADMINISTRATION

Sofosbuvir Tablets

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 sample solution should correspond to the peak in the chromatogram obtained with the reference standard solution of Sofosbuvir.

2. Dissolution Test: Sofosbuvir (As per US FDA)

2.1 Dissolution Parameter:

2.1.1 Medium : 900ml 0.05 M Phosphate Buffer pH 6.8
2.1.2 Apparatus : Paddle
2.1.3 Rotation : 75 RPM
2.1.4 Temperature : 37˚C ± 0.5˚C
2.1.5 Time : 30 minutes

2.1.6. Dissolution Medium Preparation: 6.8 gm/L potassium dihydrogen orthophosphate and adjust the pH to 6.8 with sodium hydroxide

2.1.7 Standard Preparation:

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.

2.1.8. Sample preparation

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.

2.1.9. Chromatographic system:

2.1.9.1 Column: 150 X 4.6 mm (C 18)
2.1.9.2 Flow rate: 1.5 ml/min
2.1.9.3 Wave length: 263 nm
2.1.9.4 Injection volume: 20 µl
2.1.9.5 Mobile phase

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.

Mobile phase A: Buffer:Acetonitrile (90:10)
Mobile phase B: Acetonitrile:IPA (80:20)
Final Mobile phase: 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.

2.1.10. Procedure:
Inject 20 µl of standard preparation and the sample preparation separately 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%. Calculate the release of drug in the sofosbuvir tablet by using following formula:

2.1.11. Calculation:

Sofusbivir (%):

$$=\frac{Area\ of\ spl}{Area\ of\ std} \times \frac{conc.of\ std}{conc.of\ spl} \times std\ potency\ \% \times \frac{100-LOD/WC}{100}\ \ x\ 100\ \%$$

Result : Sofusbivir in %

2.1.12. Tolerence Limit: NLT 75 % of the labeled amount

3. Assay: Sofusbivir

3.1 Chromatographic Condition:

3.1.1 Column : C18
3.1.2 Temperature : 30˚c
3.1.3 Detector : 263 nm
3.1.4 Flow rate : 1.5 ml/min
3.1.5 Injection volume : 10 µl

3.2 Mobile Phase:

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.

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

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

Final Mobile phase: 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.

3.3 Diluents

Water:Acetonitrile (70:30)

3.4 Standard Preparation:

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

3.5 Sample Preparation:

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 diluet, sonicate for about 10 minutes and cool the solution to room temperature and make up the volume to 100 ml with diluents. Centrifuge the solution. Dilute 10 ml of the solution to 25 ml with diluent. Filter the solution with 0.2 microne filter paper.

3.6 Procedure:

Inject 10 µl of standard preparation and the sample preparation separately 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%. Calculate the content of drug in the sofosbuvir tablet by using following formula:

Sofosbuvir per tablet:

$$=\frac{Spl\ Peak\ Area}{Std\ Peak\ Area} \times \frac{conc.of\ std}{conc.of\ spl} \times std\ potency\ \% \times \frac{100-LOD/WC}{100}\ \ x\ Average\ weight$$