DEPARTMENT OF DRUG ADMINISTRATION

National Medicines Laboratory

ANALYTICAL METHOD VALIDATION COMMITTEE

Sofosbuvir & Ledipasvir Tablet

Analytical Profile No.: SOF LED 075/076/AP042

Sofosbuvir and Ledipasvir tablet contains not less than 90 % and not more than 110 % of Sofosbuvir and Ledispasvir of the stated amount.

1. Identification:

1.1. Sofosbuvir: In the assay, the principle peak in the chromatogram obtained with the test solution should correspond to the peak in the chromatogram obtained with the reference solution of Sofosbuvir.

1.2. Ledipasvir: In the assay, the principle peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution of Ledipasvir.

Tests:

2. Dissolution: *Determine by Liquid Chromatography*

2.1 Dissolution Parameter

Apparatus:	Paddle
Medium:	900 ml, 1.5 % Polysorbate 80 in 0.01 M Potassium phosphate buffer with 0.0075 mg/ml butylated hydroxytoulene (BHT), adjust pH 6.0 with sodium hydroxide
Speed and Time:	75 rpm for 45 minutes
Temperature:	37 ± 0.5 ℃

Withdraw a suitable volume of the medium and filter

2.2 Test Solution: Dilute 5 ml of the filtrate to 10 ml with solvent mixture. Filter the resulting solution through 0.2-micron membrane filter.

2.3 Reference Solution: Weigh and transfer 44.4 mg of Sofosbuvir & 10 mg of Ledipasvir (20 mg with co-povidone 1:1) reference standard in 100 ml volumetric flask. Add 70 ml of solvent mixture, sonicate to dissolve and make up to the mark with same solvent. Further dilute 5 ml of this solution to 10 ml with dissolution medium. Filter it through 0.2 micron membrane filter.

2.4 Chromatographic Condition:

Column: C18 (15 cm X 4.6 mm), 5µm
Flow rate: 1.0 ml/min
Injection volume: 20 µl
Wavelength: 245 nm
Column temperature: 30℃
Detector: UV

Mobile Phase A: Acetonitrile
Mobile Phase B: 0.01 N dibasic sodium phosphate buffer, adjust pH to 6.5 with Phosphoric acid.
Solvent Mixture: Acetonitrile: Mobile phase B (65:35)

Use gradient programming as given below:

Time (min)	Mobile Phase A (% v/v)	Mobile Phase B (% v/v)
0	10	90
14	90	10
15	10	90
20	10	90

2.5 Procedure: Inject the reference solution five times as per above mentioned chromatographic condition. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, tailing factor is not more than 2.0 and the relative standard deviation for replicate

injections in not more than 2.0 %. Resolution between Sofosbuvir and Ledipasvir should be not less than 2.0. Inject test solution, measure the peak responses.

Calculate the percent release of Sofosbuvir and Ledipasvir in each tablet.

2.6 Limit: NLT 75 % (D) of the stated amount.

3. Assay: *Determine by Liquid chromatography*

3.1 Test Solution: Weigh individually 20 tablets & crush them into fine powder. Weigh accurately the powder eq. to 400 mg of Sofosbuvir into 50 ml volumetric flask, add 30 ml of methanol & sonicate for 15 minutes to dissolve with intermittent shaking. After sonication, dilute to 50 ml with same solvent. Further dilute 5 ml of the solution into 50 ml volumetric flask and dilute up to the mark with mobile phase. Filter the solution through 0.2 µm membrane filter.

3.2 Reference Solution: Weigh accurately about 45 mg of Ledipasvir (90 mg with co-povidone 1:1) reference standard into 25 ml volumetric flask. Dissolve with methanol by sonication for about 5 minutes. Further dilute 5 ml of this solution in 50 ml volumetric flask which is already containing 40 mg of Sofosbuvir reference standard dissolved with mobile phase. Filter it through 0.2 micron membrane filter.

3.3 Chromatographic Condition:

Column:	C18 (25 cm X 4.6 mm), 5µm
Injection volume:	10 µl
Flow rate:	1.0 ml/min
Wavelength:	247 nm
Detector:	UV
Column Temperature:	30 °C

Mobile Phase: A mixture of 45 volumes of Solution A & 55 volumes of Solution B, pH adjusted to 2.0 with Triethylamine

Solution A: 0.5 ml trifluoroacetic acid in 1000 ml of acetonitrile

Solution B: 0.5 ml trifluoroacetic acid in 1000 ml of methanol

3.4 Procedure: Inject the 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, tailing factor is not more than 2.0, the relative standard deviation for replicate injections in not more than 2.0 % and the resolution between Sofosbuvir and Ledipasvir is not less than 2%.

Calculate the content of Sofosbuvir and Ledipasvir in each tablet.

4. Other Test: As per pharmacopoieal requirement.