ANALYTICAL METHOD VALIDATION COMMITTEE FOR NON PHARMACOPOEIAL PRODUCT

DEPARTMENT OF DRUG ADMINISTRATION

National Medicines Laboratory

Sofosbuvir & Ledipasvir Tablet

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

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

1. Identification:

1.1. Sofosbuvir:

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.

1.2. Ledipasvir:

In the assay, the principle peak in the chromatogram obtained with the sample solution corresponds to the peak in the chromatogram obtained with the reference standard solution of Ledipasvir.

2. Dissolution: Determine by thin layer chromatography

2.1 Dissolution Parameter

Apparatus: Paddle

Medium: 900ml, 1.5% Polysorbate 80 in 0.01 molar Potassium phosphate buffer with 0.0075 mg/ml butylated hydroxytoulene (BHT), adjust pH 6.0

Speed and Time: 75 rpm for 45 minutes

Temperature: 37+/-0.5℃

2.2 Chromatographic Condition:

Column: C18 (15 cm X 4.6 mm), 5µm

Injection volume: 20 µl

Flow rate: 1.0 ml/min

Wavelength: 245 nm

Column temperature: 30°C

Detector: UV

Mobile Phase A: Acetonitrile

Mobile Phase B: 0.01N dibasic sodium phosphate buffer, adjust pH to 6.5 with phosphoric acid.

Solvent Mixture: Acetonitrile: Mobile phase B (65:35)

Gradient programme using the conditions 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

2.3 Test Solution:

Withdraw a suitable volume of medium and filter. Dilute 5 ml of the filtrate to 10ml with solvent mixture. Filter it through 0.2-micron membrane filter.

2.4 Reference Solution:

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

2.5 Procedure:

Inject the reference solution. 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 test solution, measure the peak responses.

Calculate the percent release of Sofosbuvir and Ledipasvir.

2.6 Limit:

D. NLT 75% of the stated amount

3. Assay: Determine by thin layer chromatography

3.1 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 0.5ml trifluoroacetic acid in 1000ml of acetonitrile

Solution B 0.5ml trifluoroacetic acid in 1000ml of methanol

2.2 Test Solution:

Weigh individually 20 tablets & crush the tablet 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 5ml of the solution into 50ml volumetric flask and dilute up to the mark with mobile phase. Filter the solution through 0.2 µm membrane filter.

2.3 Reference Solution:

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

2.4 Procedure:

Inject the reference solution five/six times and sample solutions. 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.

3. Other Test: As per pharmacopoieal requirement.