Government of Nepal
Ministry of Health and Population
Department of Drug Administration
National Medicines Laboratory
Quality and Method Validation Section

Analytical profile of Sofosbuvir & Ledipasvir Tablet

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

1. Identification:

1.1. Sofosbuvir:

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.

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.

2. Dissolution (Sofosbuvir & Ledipasvir)

2.1 Dissolution Parameters:

Apparatus: Paddle

Medium: 900ml of 1.5% Polysorbate 80 in 0.01 molar Potassium phosphate buffer (1.36g in 1000ml) with 0.0075 mg/ml butylated hydroxytoulene (BHT), adjust pH 6.0 with Sodium hydroxide.

Speed and Time: 75 rpm and 45 minutes

Withdraw a suitable volume of the medium and filter.

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

2.3 Test Solution: Dilute 5 ml of the filtrate to 10ml with solvent mixture.

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.

2.5 Chromatographic Condition:

Column: C18 (15 cm X 4.6 mm), 5µm

Flow rate: 1.0 ml/min

Wavelength: UV 245 nm

Injection volume: 20 µl

Mobile Phase A: Acetonitrile

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

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**

2.6 Procedure: Inject the reference solution five/six times and test 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. Measure the peak response. Calculate the percent release of Sofosbuvir and Ledipasvir.

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

3. Assay (Estimation of Sofosbuvir & Ledipasvir)

3.1 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.

3.2 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.

3.3 Chromatographic Condition:

Column: C18 (25 cm X 4.6 mm), 5µm

Flow rate: 1.0 ml/min

Wavelength: UV 247 nm

Injection volume: 10 µl

Column Oven 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

3.4 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. Measure the peak response. Calculate the content of Sofosbuvir and Ledipasvir.

4. Other tests: As per pharmacopoeial requirement.