DEPARTMENT OF DRUG ADMINISTRATION
National Medicines Laboratory
ANALYTICAL METHOD VALIDATION COMMITTEE

Daclatasvir Tablets

Analytical Profile No.: DAC 075/076/AP 040

Daclatasvir tablet contains not less than 90 % and not more than 110 % of the stated amount of Daclatasvir.

1. **Identification:** 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 Daclatasvir.

Tests:

2. Dissolution: *Determine by Liquid Chromatography*

2.1 Dissolution Parameters:

Apparatus: Paddle
Medium: 1000 ml of Phosphate buffer pH 6.8
Speed and time: 75 rpm and 45 minutes
Temperature: 37 ± 0.5 ^{0}C

Withdraw a suitable volume of the medium and filter.

2.2 Test Solution: Dilute 1 ml of filtrate to 20 ml with methanol. Filter the resulting solution through 0.2 µm membrane filter.

2.3 Reference Solution: Weigh accurately about 33.0 mg Daclatasvir dihydrochloride reference standard in 100 ml volumetric flask. Add about 70 ml of dissolution medium, sonicate to dissolve and make up the volume to 100 ml with dissolution medium. Dilute 5 ml of resulting solution to 50 ml with dissolution medium. Further dilute 2 ml of this solution to 20 ml with methanol. Filter the final solution through 0.2 µm membrane filter.

2.4 Chromatographic system and Procedure: Proceed as directed under the Assay

Calculate the % release of Daclatasvir per tablet.

2.5 Limit: NLT 80 % (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 30 mg of Daclatasvir in 100 ml volumetric flask, add 70 ml of methanol, sonicate to dissolve with intermittent shaking at 20°C to 25°C temperature and make volume to 100 ml with same solvent. Stir for 10 minutes. Dilute 5 ml of resulting solution to 50 ml with same solvent. Further dilute 10 ml of this solution to 25 ml with same solvent. Filter the final solution through 0.2 µm membrane filter.

3.2 Reference Solution: Weigh accurately about 33.0 mg Daclatasvir dihydrochloride reference standard in 100 ml volumetric flask. Add about 70 ml of methanol and sonicate for about 5 minutes and make up the volume to 100 ml with same solvent. Dilute 5 ml of resulting solution to 50 ml with methanol. Further dilute 10 ml of this solution to 25 ml with methanol. Filter the final solution through 0.2 µm membrane filter.

3.3 Chromatographic system:

Column: C18, 250*4.6 mm, 5 µm
Injection volume: 5 µl
Flow rate: 1.0 ml/min
Column Temperature: 25°C
Detector: UV 315 nm
Mobile Phase: Methanol: Water (98:02)

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 and the relative standard deviation for replicate injections is not more than 2.0 %. Inject the test solution. Measure the peak responses. Calculate the content of Daclatasvir per tablet.

4. Other tests: As per pharmacopoieal requirement.