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

Analytical profile of S (-) Amlodipine & Hydrochlorothiazide Tablets

Analytical Profile No.: Amlo Hydro 076/77/AP 076

S (-) Amlodipine & Hydrochlorothiazide Tablets contains not less than 90.0% and not more than 110.0% of the stated amount of S (-) Amlodipine & Hydrochlorothiazide.

1. Identification:

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.

Tests:

2. Dissolution: *Deteremine by liquid chromatography*

2.1 Dissolution Parameters:

Apparatus: Paddle

Medium: 900ml of 0.1N HCl.

Speed and Time: 75 rpm and 45 minutes

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography.

2.2 Test Solution: Dilute 5 ml of the filtrate to 25 ml with mobile phase.

2.3 Reference Solution:

For S-amlodipine 2.5 mg & Hydrochlorothiazide 12.5 mg

Weigh accurately about 20 mg of S (-) Amlodipine WS & 70 mg of Hydrochlorothiazide WS in 500 ml volumetric flask. Add about 350 ml of dissolution media, sonicate for about 10-15 min, cool to room temperature and make up the volume with dissolution media. Dilute 5 ml of the solution to 50 ml with dissolution medium, mix well. Further dilute 5 ml of the solution to 25 ml with mobile phase.

For S-amlodipine 5 mg & Hydrochlorothiazide 12.5 mg

Weigh accurately about 15 mg of S (-) Amlodipine WS & 30 mg of Hydrochlorothiazide WS in 200 ml volumetric flask. Add about 150 ml of dissolution media, sonicate for about 10-15 min, cool to room temperature and make up the volume with dissolution media. Dilute 5 ml of the

solution to 50 ml with dissolution medium, mix well. Further dilute 5 ml of the solution to 25 ml with mobile phase.

2.4 Procedure: Use the chromatographic system as described in the Assay except injection volume is 100µl.

Inject the reference solution and the test solution.

2.5 Limit: Not less than 70 percent (D) of the stated amount of S (-) Amlodipine & Hydrochlorothiazide.

3. Uniformity of Content

Determine by liquid chromatography, as described in the Assay, using the following solution as the test solution.

Test Solution: Place a tablet in a 50ml volumetric flask, add 30ml of mobile phase, sonicate for 20 minutes to disperse whole tablet. Cool, make up the volume to 100 ml with same solvent and mix well. Further dilute 5 ml of the solution to 25 ml with same solvent.

4. Assay: *Determine by liquid chromatography*

4.1 Test Solution: Weigh and transfer intact tablets equivalent to 50 mg of Hydrochlorothiazide to a 100ml volumetric flask. Add about 70 ml of mobile phase and sonicate for about 20 mins to disperse tablets, cool to room temperature and make up the volume to 100 ml with same solvent. Dilute 5 ml of this solution to 50 ml with mobile phase.

4.2 Reference Solution:

For S-amlodipine 2.5 mg & Hydrochlorothiazide 12.5 mg

Weigh accurately about 15 mg of S (-) Amlodipine Besilate WS & 50 mg of Hydrochlorothiazide WS in 100 ml volumetric flask. Add about 70 ml of mobile phase and sonicate for about 10-15 mins to dissolve, cool to room temperature and make up the volume to 100 ml with same solvent. Dilute 5 ml of this solution to 50 ml with mobile phase.

For S-amlodipine 5 mg & Hydrochlorothiazide 12.5 mg

Weigh accurately about 30 mg of S (-) Amlodipine Besilate WS & 50 mg of Hydrochlorothiazide WS in 100 ml volumetric flask. Add about 70 ml of mobile phase and sonicate for about 10-15

mins to dissolve, cool to room temperature and make up the volume to 100 ml with same solvent. Dilute 5 ml of this solution to 50 ml with mobile phase.

4.3 Chromatographic system:

- **Column:** C8, 25 cm x 4.6 mm, 5µm particle size
- **Flow rate:** 0.8 ml/min
- **Wavelength:** 237 nm
- **Injection volume:** 20 µl
- **Detector:** UV/PDA
- **Column temperature:** Ambient
- **Tray Temperature:** 4-8ºC
- **Mobile Phase:** A mixture of 60 volumes of buffer solution and 40 volumes of Acetonitrile. To this add 5 ml of Triethylamine. Mix well and adjust the pH to 7.1 using glacial acetic acid.
- **Buffer solution:** Prepare 0.1M Ammonium Acetate solution containing 0.005M of 1-Octane sulphonic acid sodium salt.

4.4 Procedure: Inject the reference solution five times. 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 S (-) Amlodipine & Hydrochlorothiazide in tablet.

5. Other tests: As per pharmacopoeial requirements.