DEPARTMENT OF DRUG ADMINISTRATION
National Medicines Laboratory
ANALYTICAL METHOD VALIDATION COMMITTEE

S (-) Amlodipine and Hydrochlorothiazide Tablets

Analytical Profile No.: Amlo Hydro 076/077/AP076

S (-) Amlodipine and Hydrochlorothiazide Tablets contain not less than 90 per cent and not more than 110 per cent of the stated amount of S (-) Amlodipine and Hydrochlorothiazide.

1. Identification:

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 S (-) Amlodipine and Hydrochlorothiazide.

Tests:

2. Dissolution: *Determine by Liquid Chromatography*

2.1 Dissolution Parameters:

Apparatus: Paddle

Medium: 900 ml of 0.1 N HCl

Speed and time: 75 rpm and 45 minutes

Withdraw the suitable volume of the medium and filter.

2.2 Test Solution:

Filter the withdrawn solution through Whatmann filter paper No. 41 and dilute 5 ml of the filtrate to 25 ml with mobile phase. Filter the resulting solution through 0.45 µ nylon filter paper.

2.3 Reference Solution:

For S-amlodipine 2.5 mg and Hydrochlorothiazide 12.5 mg

Weigh accurately about 20 mg of S (-) Amlodipine Besilate and 70 mg of Hydrochlorothiazide WS in to a 500 ml clean and dry volumetric flask. Add about 350 ml dissolution media, sonicate

for about 10-15 minutes and make up the volume with same. Dilute 5 ml of the solution to 50 ml with dissolution media and mix well. Further dilute 5 ml of this solution to 25 ml with mobile phase. Filter the solution through 0.45 µ nylon filter paper.

For S-amlodipine 5 mg and Hydrochlorothiazide 12.5 mg

Weigh accurately about 15 mg of S (-) Amlodipine Besilate and 30 mg of Hydrochlorothiazide WS in to a 200 ml clean and dry volumetric flask. Add about 150 ml dissolution media, sonicate for about 10-15 minutes and make up the volume with same. Dilute 5 ml of the solution to 50 ml with dissolution media and mix well. Further dilute 5 ml of this solution to 25 ml with mobile phase. Filter the solution through 0.45 µ nylon filter paper.

2.4 Chromatographic system:

Proceed as directed for the assay use 100 µl as injection volume

2.5 Procedure:

Inject 100 µl of standard preparation five times. 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 %. After the completion of the system suitability test parameter, inject 100 µl of each of the sample solution separately. Inject blank solution to check any interference and perform bracketing of standard preparation after injecting test solution.

Calculate the percentage release of S (-) Amlodipine and Hydrochlorothiazide in each tablet at specified time.

2.6 Limit:

Not less than 70 % (D) of the labeled amount of S (-) Amlodipine Besilate equivalent to S (-) Amlodipine and Hydrochlorothiazide.

3. Content Uniformity: *Determine by Liquid Chromatography*

3.1 Test solution:

Transfer individual tablet to a 50 ml volumetric flask, add 30 ml of mobile phase and sonicate for 20 minutes. Cool to room temperature and make up to volume with same and mix well. Filter the solution through Whatmann filter paper No. 1. Dilute 5 ml of the filtrate to 25 ml with mobile phase. Filter the final solution through 0.2 µm membrane filter.

3.2 Reference solution:

Proceed as directed under assay.

3.3 Chromatographic system and Procedure:

Proceed as directed under assay.

Calculate the content of S (-) Amlodipine and Hydrochlorothiazide in each tablet.

3.4 Limit: 85 - 115% of the stated amount

4. Assay: *Determine by Liquid Chromatography*

4.1 Test solution:

Weigh and transfer intact tablets equivalent to 50 mg of Hydrochlorothiazide to a 100 ml volumetric flask; add 80 ml mobile phase and sonicate for 20 minutes. Cool the solution to room temperature, make up to mark with the same solvent and mix well. Filter the solution through Whatmann filter paper No.1. Dilute 5 ml of the filtrate to 50 ml with mobile phase. Filter the final solution through 0.2 µm membrane filter.

4.2 Reference solution:

For S-amlodipine 2.5 mg and Hydrochlorothiazide 12.5 mg

Weigh accurately about 15 mg of S (-) Amlodipine Besilate and 50 mg Hydrochlorothiazide WS in 100 ml volumetric flask, add 80 ml of mobile phase, mix well by sonicating for about 10-15 minutes and make up the volume using the same solvent. Transfer 5 ml of the resulting solution in 50 ml volumetric flask and make up the volume using mobile phase. Filter the final solution through 0.2 µm membrane filter.

For S-amlodipine 5 mg and Hydrochlorothiazide 12.5 mg

Weigh accurately about 30 mg of S (-) Amlodipine Besilate and 50 mg Hydrochlorothiazide WS in 100 ml volumetric flask, add 80 ml of mobile phase, mix well by sonicating for about 10-15 minutes and make up the volume using the same solvent. Transfer 5 ml of the resulting solution in 50 ml volumetric flask and make up the volume using mobile phase. Filter the final solution through 0.2 µm membrane filter.

4.3 Chromatographic system:

Column:	a stainless steel column 25 cm x 4.6 mm, packed with octyl silane bonded to porous silica (5 µm)
Flow rate:	0.8 ml per minute
Wavelength:	237 nm
Injection volume:	20 µl
Column temperature:	ambient
Detector:	UV
Tray temperature:	4-8 ^{0} C
Mobile phase:	a mixture of 60 volumes of buffer solution and 40 volumes of acetonitrile. To this add 5 ml triethylamine, mix well and adjust the pH to 7.1 using glacial acetic acid.
Buffer:	0.1 M ammonium acetate solution containing 0.005 M of 1-octane sulfonic acid sodium salt

4.4 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 is not more than 2.0 per cent. Inject the reference solution and the test solution. Calculate the content of S (-) Amlodipine and Hydrochlorothiazide in each tablet.

5 Other tests: As per pharmacopoeial requirements.