DEPARTMENT OF DRUG ADMINISTRATION
National Medicines Laboratory
ANALYTICAL METHOD VALIDATION COMMITTEE

Frusemide & Spironolactone Tablets

Analytical Profile No.: Fru Spiro 078/079/AP 098

Frusemide & Spironolactone Tablets contains not less than 90.0% and not more than 110.0% of the stated amount of Frusemide & Spironolactone.

Usual Strength: Frusemide 20 mg & Spironolactone 50 mg

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 For Frusemide

2.1.1 Dissolution Parameters:

Apparatus: Paddle

Medium: 900ml of phosphate buffer pH 5.8 (Dissolve 6.8g of Potassium dihydrogen phosphate in 1000ml water & adjust pH 5.8 with 0.2N Sodium hydroxide)

Speed and Time: 50 rpm and 45 minutes

Withdraw a suitable volume of the medium and filter.

2.1.2 Test Solution: Dilute the filtrate, if necessary with dissolution medium.

2.1.3 Reference Solution: Weigh accurately about 22.2 mg of Frusemide WS in 100 ml volumetric flask. Add about 70 ml of methanol, sonicate to dissolve, cool to room temperature and make up the volume with same solvent. Further dilute 5 ml of this solution to 50 ml with dissolution medium.

2.1.4 Procedure: Use the chromatographic system as described in the Assay using 20 µl injection volume.

Inject the reference solution and the test solution.

Calculate the content of Frusemide.

2.1.5 Limit: Not less than 75 percent (D) of the stated amount of Frusemide.

2.2 For Spironolactone

2.2.1 Dissolution Parameters:

Apparatus: Paddle

Medium: 1000ml of 0.1N HCl containing 0.1% Sodium Lauryl Sulphate

Speed and Time: 75 rpm and 60 minutes

Withdraw a suitable volume of the medium and filter.

2.2.2 Test Solution: Dilute the filtrate, if necessary with dissolution medium.

2.2.3 Reference Solution: Weigh accurately about 50 mg of Spironolactone WS in 100 ml volumetric flask. Add about 70 ml of methanol, sonicate to dissolve, cool to room temperature and make up the volume with same solvent. Further dilute 5 ml of this solution to 50 ml with dissolution medium.

2.2.4 Procedure: Use the chromatographic system as described in the Assay using 20 µl injection volume.

Inject the reference solution and the test solution.

Calculate the content of Spironolactone.

2.2.5 Limit: Not less than 75 percent (D) of the stated amount of Spironolactone.

3. Assay: *Determine by liquid chromatography*

3.1 Solvent Mixture: Equal volumes of water & acetonitrile

3.2 Test Solution: Weigh and powder 20 tablets. Weigh a quantity of powder equivalent to 1 tablet into 100ml volumetric flask, add about 70 ml of solvent mixture, sonicate with intermittent shaking, cool and make volume to 100 ml with same solvent. Further dilute 5 ml of this solution to 50 ml with same solvent.

4.2 Reference Solution: Weigh accurately about 20 mg of Frusemide WS and 50 mg of Spironolactone WS in 100 ml volumetric flask. Add about 70 ml of solvent mixture, sonicate 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 same solvent.

4.3 Chromatographic system:

- **Column:** C18, (150 x 4.6 mm), 5 µ particle size
- **Flow rate:** 1.0 ml/min
- **Wavelength:** 254 nm
- **Column Oven Temperature:** 30 ºC
- **Injection volume:** 10 µl

- **Detector:** UV
- **Mobile Phase:** A mixture of 50 volumes of buffer and 50 volumes of Acetonitrile
- **Buffer:** Acetate buffer pH 4.0 (7.7190 gm Sodium acetate anhydrous in 1000ml water , pH 4.0 adjust with acetic acid)

4.4 Procedure: Inject the reference solution five 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 is not more than 2.0% and unless the resolution between Frusemide & Spironolactone is not less than 2. Measure the peak responses. Calculate the content of Frusemide & Spironolactone in the tablets.

5. Other tests: As per pharmacopoeial requirements.