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

Analytical Profile of Sulphamethoxazole & Trimethoprim Powder (vet)

Analytical Profile No.: Cotri 081/082/AP 171

Sulphamethoxazole & Trimethoprim Powder contains not less than 92.5% and not more than 107.5% of the stated amount of Sulphamethoxazole and Trimethoprim.

Usual Strength: Trimethoprim (8%w/w) and Sulphamethoxazole (40% w/w)

1. Identification:

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

2. **Assay:** *Determine by liquid chromatography*

2.1 Test solution: Weigh the powder equivalent to 160 mg of Sulphamethoxazole in a 100 ml dry volumetric flask, add 50 ml of methanol, and sonicate for 5 minutes to dissolve with intermittent shaking. Cool the sample solution to room temperature then, make up the volume with methanol and mix. Dilute 5 ml of the solution to 50 ml with mobile phase and mix.

2.2 Reference solution:

2.2.1 Standard Stock Solution A (Trimethoprim): Weigh accurately about 16 mg of Trimethoprim WS and dissolve in 50 ml of methanol with the aid of ultrasound for 5 minutes.

2.2.2 Standard Stock Solution B (Sulphamethoxazole): Weigh accurately about 40 mg of Sulphamethoxazole WS and dissolve in 25 ml of methanol with the aid of ultrasound for 5 minutes.

2.2.3 Reference Solution: Dilute 5 ml of each standard stock solution A and B to 50 ml with mobile phase.

2.3 Chromatographic system:

Column: C18 (150mm X 4.6mm, 5µm)
Flow rate: 1.0 ml/min
Wavelength: 254 nm
Injection volume: 20 µl
Column Temperature: Ambient
Mobile phase: A mixture of 700 ml of water, 200 ml of acetonitrile, and 1 ml of triethylamine, adjusted to pH 5.9 with 0.2 M Sodium hydroxide or dilute glacial acetic acid (1.0%v/v) and dilute to 1000 ml water

2.4 Procedure: Inject the reference solution five times and test the solutions. 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%. Measure the peak responses. Calculate the content of Trimethoprim and Sulphamethoxazole.

3. Other tests: As per Pharmacopoeial requirements.