DEPARTMENT OF DRUG ADMINISTRATION
National Medicines Laboratory
ANALYTICAL METHOD VALIDATION COMMITTEE

Metronidazole & Diloxanide Furoate tablet

Analytical Profile No.: Metr Dilo 075/076/AP013

Metronidazole and Diloxanide Furoate tablet contains not less than 90 per cent and not more than 110 per cent of the stated amount of Metronidazole and Diloxanide Furoate.

1. Identification:

1.1. Metronidazole: 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 Metronidazole.

1.2. Diloxanide Furoate: 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 Diloxanide Furoate.

Tests:

2. Dissolution (Metronidazole): *Determine by Liquid Chromatography*

2.1 Dissolution Parameter:

Apparatus:	Paddle
Medium:	900 ml 0.1 N HCl
Speed and time:	100 rpm and 60 minutes
Temperature:	37˚C ± 0.5˚C

Withdraw the suitable volume of the medium and filter.

2.2 Test Solution: Dilute 5 ml of the withdrawn solution to 50 ml with mobile phase and filter through 0.2 µm membrane filter.

2.3 Reference Solution: Weigh accurately about 44.4 mg Metronidazole reference standard in 100 ml volumetric flask. Add about 70 ml of dissolution medium and sonicate for about 10 minutes

and make up the volume to 100 ml with dissolution medium. Dilute 5 ml of resulting solution to 50 ml with mobile phase and filter through 0.2 µm membrane filter.

2.4 Chromatographic system: Use the chromatographic system as described in assay

2.5 Procedure: Inject 20 µl of standard preparation five times using 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 in not more than 2.0 %. After the completion of the system suitability test parameter, inject 20 µl of each of the sample solution separately. Measure the peak responses and calculate the per cent release of the drug.

2.6 Limit: Not less than 80 per cent (D) of the stated amount.

3. Assay: *Determine by Liquid Chromatography*

3.1 Test solution: Weigh individually 20 tablets and crush the tablet into fine powder. Weigh accurately the powder eq. to 100 mg of Metronidazole in 100 ml volumetric flask, add 70 ml of mobile phase and sonicate for 15 minutes to dissolve. After sonication, cool to room temperature and make volume to 100 ml with mobile phase. Filter and dilute 5 ml of resulting solution to 50 ml with mobile phase. Filter the final solution through 0.2 µm membrane filter.

3.2 Reference solution: Weigh accurately about 100 mg Metronidazole reference standard and 125 mg Diloxanide furoate reference standard in 100 ml volumetric flask. Add about 70 ml of mobile phase and sonicate for about 10 minutes, cool to room temperature and make up the volume to 100 ml with mobile phase. Dilute 5 ml of resulting solution to 50 ml with mobile phase. Filter the final solution through 0.2 µm membrane filter.

3.3 Chromatographic system:

Column:	C18 (25 cm x 4.6 mm), 5 µm
Flow rate:	1.0 ml per minute
Wavelength:	241nm

Injection Volume: 20 µl

Detector: UV

Column temperature: 30°C

Mobile phase: a mixture of 20 volumes of acetonitrile, 50 volumes of methanol and 30 volumes of phosphate buffer prepared by mixing 1.625 g KH_2PO_4 and 0.300 g K_2HPO_4 in 550ml water.

3.4 Procedure: Inject the reference solution and the test solution. 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 resolution between two peaks should be not less than 2. Measure the peak responses. Calculate the content of Metronidazole and Diloxanide furoate per tablet.

4. Other tests: As per pharmacopoeial requirements.