

**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 sample solution should correspond to the peak in the chromatogram obtained with the reference standard solution of Metronidazole.

**1.2. Diloxanide Furoate**

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 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.

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**2.2 Chromatographic system:**

Use the chromatographic system as described in assay

**2.3 Test Solution:**

Dilute 5 ml of the withdrawn solution to 50 ml with mobile phase and filter through 0.2  $\mu\text{m}$  membrane filter.

**2.4 Reference Solution:**

Weigh accurately about 44.4 mg Metronidazole working 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  $\mu\text{m}$  membrane filter.

**2.5 Procedure:**

Separately inject 20  $\mu\text{l}$  of standard, sample solution and blank solution (dissolution medium) and obtain the respective chromatograms. 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%. Measure the peak responses and calculate the per cent release of the drug.

**2.6 Limit:**

D. Not less than 80 per cent of the stated amount.

**3. Assay:** Determine by Liquid Chromatography

**3.1 Chromatographic system:**

- **Column:** C18 (25 cm x 4.6 mm), 5  $\mu\text{m}$
- **Flow rate:** 1.0 ml per minute
- **Wavelength:** 241nm

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- **Injection Volume:** 20ul
- **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  $\text{KH}_2\text{PO}_4$  and 0.300 g  $\text{K}_2\text{HPO}_4$  in 550ml water.

**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 5ml of resulting solution to 50 ml with mobile phase. Filter the final solution through 0.2  $\mu\text{m}$  membrane filter.

**3.2 Reference solution:**

Weigh accurately about 100 mg Metronidazole working standard and 125 mg Diloxanide furoate working 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 5ml of resulting solution to 50ml with mobile phase. Filter the final solution through 0.2  $\mu\text{m}$  membrane filter.

**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:**

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As per pharmacopoeial requirements.