

**DEPARTMENT OF DRUG ADMINISTRATION**

**National Medicines Laboratory**

**ANALYTICAL METHOD VALIDATION COMMITTEE**

**Brimonidine Tartrate & Timolol Maleate Eye Drops**

**Analytical Profile No.:** Brim Timil 078/079/AP 097

Brimonidine Tartrate & Timolol Maleate Eye Drops contains not less than 90.0% and not more than 110.0% of the stated amount of Brimonidine Tartrate & Timolol Maleate.

**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.

**2. pH:** As per manufacturer's specification

**3. Particulate Matter:** As per IP latest edition

**4. Sterility:** As per IP latest edition

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

**5.1 Test solution:** Dilute 10 ml of the ophthalmic solution to 100 ml with water.

**5.2 Reference solution:** Weigh accurately about 40 mg of Brimonidine Tartrate WS and 136 mg of Timolol Maleate WS and transfer into 200 ml volumetric flask. Add about 100 ml of water, and sonicate, cool at room temperature and make up the volume to 200 ml with same solvent.

**5.3 System Suitability Solution:** A mixture of 0.02% w/v solution of Brimonidine Tartrate and 0.002% w/v solution of Maleic acid.

**5.4 Chromatographic system:**

- **Column:** C18, (250 x 4.6 mm), 5  $\mu$  particle size
- **Flow rate:** 1.2 ml/min
- **Wavelength:** 295 nm
- **Injection volume:** 20  $\mu$ l
- **Detector:** UV
- **Column temperature:** 40 °C
- **Mobile Phase:** A mixture of 65 volumes of buffer and 35 volumes of Methanol
  - **Buffer:** Dissolve 11.5 gm of Sodium dihydrogen phosphate in 1000ml of water. Adjust pH to  $2.8 \pm 0.05$  with dilute phosphoric acid

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**5.5 Procedure:** Inject the system suitability solution. The test is not valid unless the resolution between Maleic acid and Brimonidine Tartrate is NLT 2.0. Inject the reference solution five times. 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%. Inject the test solution. Measure the peak responses. Calculate the content of Brimonidine Tartrate and Timolol Maleate in eye drops.

**6. Other tests:** As per pharmacopoeial requirements.

Subject to approval from DAC