DEPARTMENT OF DRUG ADMINISTRATION

National Medicines Laboratory

ANALYTICAL METHOD VALIDATION COMMITTEE

Tropicamide and Phenylephrine HCl Ophthalmic Solution

Analytical Profile No.: Trop Phen 075/076/AP038

Tropicamide and Phenylephrine HCl Ophthalmic Solution contains not less than 90 % and not more than 110 % of the stated amount of Tropicamide and not less than 90 % and not more than 110 % of the stated amount of Phenylephrine HCl.

1. Identification:

1.1 Tropicamide: 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.

1.2 Phenylephrine HCl: 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. pH: 4.0 to 5.8

3. Extractable Volume: The average content of the 3 containers is not less than the nominal volume and not more than 5.45 ml of the nominal volume.

4. Particulate matter (By Light Obscuration Particle Counter):

As per Indian Pharmacopoeia (latest edition)

5. Sterility test: As per Indian Pharmacopoeia (latest edition)

6. Assay: *Determine by Liquid Chromatography*

6.1 Test Solution: Transfer an accurately measured volume of ophthalmic solution, equivalent to about 16 mg of Tropicamide (i.e. 2 ml) to a 100 ml volumetric flask, add 2 ml of dilute sulphuric acid (1 in 6), sonicate and make up the volume with mobile phase. Dilute 5 ml of this solution to 50 ml with mobile phase. Filter the resulting solution through 0.2 µm membrane filter.

6.2 Reference Solution:

6.2.1 Reference Solution A: Weigh accurately about 40 mg of Tropicamide reference standard and transfer into 50 ml volumetric flask. Dissolve it with 2 ml of dilute sulphuric acid (1 in 6), sonicate and make up the volume with mobile phase.

6.2.2 Reference Solution B: Weigh accurately about 50 mg of Phenylephrine HCl reference standard and transfer into 50 ml volumetric flask. Dissolve it with 10 ml of mobile phase, add 10 ml of **Reference Solution A** and make up the volume with mobile phase. Dilute 5 ml of this solution to 50 ml volumetric flask with mobile phase. Filter the resulting solution through 0.2 µm membrane filter.

6.3 Chromatographic system:

Column: C18, (250 × 4.6) mm; 5 µm
Flow rate: 1.2 ml/ min.
Wavelength: 257 nm
Injection volume: 10 µl
Column temperature: 40 ºC
Detector: UV
Mobile phase: Dissolve 1.1 g of sodium 1-octane sulphonate in 1000 ml of solution A
Solution A: A mixture of methanol and water (58:42), pH adjusted to 3.0 with 3M orthophosphoric acid.

6.4 Procedure: Inject the Reference solution B five times as per above mentioned chromatographic conditions. The test is not valid unless the resolution between Phenylephrine and Tropicamide is not less than 2, 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 Tropicamide and Phenylephrine HCl in Ophthalmic Solution.

7. Other tests: As per pharmacopoeial requirements.