

**ANALYTICAL METHOD VALIDATION COMMITTEE FOR NON  
PHARMACOPOEIAL PRODUCT  
DEPARTMENT OF DRUG ADMINISTRATION  
National Medicines Laboratory**

## **Tropicamide and Phenylephrine HCl Ophthalmic Solution**

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

Tropicamide and Phenylephrine HCl Ophthalmic Solution contain 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.

### **2. pH:** 4.0 to 5.8

### **3. Extractable Volume**

**Procedure:** Select 3 containers, take up individually the total contents of each container selectes into a dry syringe of a capacity not exceeding three times the volume to be measured and fittes with a 21-guage needle not less than 2.5 cm (1 inch) in length. Expel any air bubbles from the syringe and needle, then discharge the contents of the syringe without emptying the needle into a standardized dry cylinder (graduated to contain rather than to deliver the designated volumes) of such size that the volume to be measured occupes at least 40 per cent of its graduated volume. Repeat the procedure until the contents of the 3 containers have been transferred and measure the 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.

**Acceptance Criteria:** 5.0 ml – 5.45 ml

### **4. Particulate matter (By Light Obscuration Particle Counter)**

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As per Indian Pharmacopoeia (latest edition)

**Limit:**

≥10 µm: 50 particles/ml

≥25 µm: 5 particles/ml

≥50 µm: 2 particles/ml

**5. Sterility test**

As per Indian Pharmacopoeia (latest edition)

**Limit:** Shows no growth of microorganisms.

**6. Assay**

**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 50ml volumetric flask with mobile phase.

**6.2 Standard Solution:**

**Standard Solution A:**

Weigh accurately about 40 mg of Tropicamide WS 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.

**Standard Solution B:** Weigh accurately about 50 mg of Phenylephrine HCl WS and transfer into 50 ml volumetric flask. Dissolve it with 10ml of mobile phase, add 10 ml of Standard Solution A and make up the volume with mobile phase. Dilute 5 ml of this solution to 50ml volumetric flask with mobile phase.

**6.3 Chromatographic system:**

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

**6.4 Procedure:** Inject the reference solution. 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%.

Calculate the content of Tropicamide and Phenylephrine HCl in Ophthalmic Solution.

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