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# Simultaneous estimation of Naphazoline HCl and Phenylephrine HCl by HPLC method in Eye Drops

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**Abstract**
A simple, rapid, accurate, precise and inexpensive reverse phase- High performance Liquid Chromatography (RP-HPLC) method was developed for simultaneous estimation of Naphazoline HCl (NAPH) and Phenylephrine HCl (PHE) utilizing isocratic separation mode with Grace C18, (250 mm X 4.6 mm i.d., 5 μm particle size) column. Optimum mobile phase consisted of Potassium Dihydrogen Phosphate buffer pH 3: Acetonitrile: Triethyleamine in the ratio of 80:20:0.01 v/v/v (pH 3 adjusted with o-Phosphoric acid) with effluent flow rate of 1 ml/min and detection wavelength at 276 nm. The column temperature was kept ambient. Naphazoline HCl and Phenylephrine HCl were found at retention time of 5.976 and 3.154 minutes respectively. Linearity was established in the range of 10-60μg/ml for NAPH and 24-144μg/ml for PHE. With correlation coefficient 0.9997 and 0.9998 respectively. The LOD and LOQ values were found to be 0.28 and 0.85μg/ml for NAPH and 0.48 and 1.47μg/ml for PHE respectively. The developed method can be used for routine quality control analysis of both drugs in eye drops formulation.

**Keywords:** Naphazoline HCl (NAPH), Phenylephrine HCl (PHE), Isocratic, RP-HPLC, Quality control, Eye drops.

## Introduction
Naphazoline HCl, chemically 2- (naphthalen- 1 -ylmethyl)-4, 5-dihydro-1H- imidazole hydrochloride (Fig.1) is a rapid acting sympathomimetic vasoconstrictor of ocular arterioles. It is α-adrenergic receptor agonist that constricts the vascular system of the conjunctiva. This effect is due to direct stimulation of the drug upon the alpha adrenergic receptors in the arterioles of the conjunctiva resulting in decreased conjunctival congestion. It belongs to the imidazoline class of sympathomimetics [1, 2].
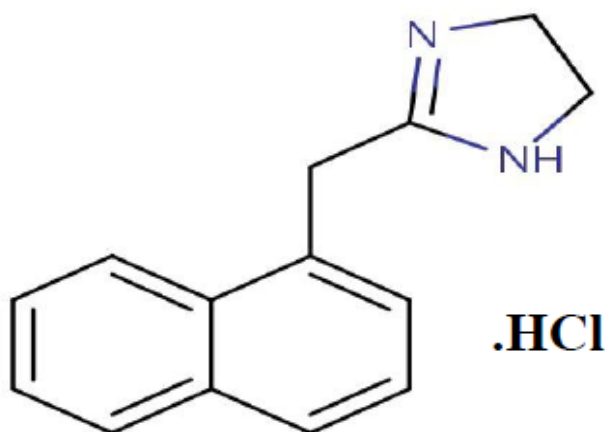


**Fig 1:** Chemical Structure of Naphazoline HCl

Phenylephrine HCl, chemically 3-[(1R)-1-hydroxy-2-(methylamino) ethyl] phenol hydrochloride (Fig. 2) is a selective α1-adrenergic receptor agonist which is used primarily as a decongestant, as an agent to dilate the pupil, and to increase blood pressure. Phenylephrine HCl produces its local and systemic actions by stimulating α1-adrenergic receptors. As results contraction of arteriolar smooth muscle in the periphery occurs [3, 4].

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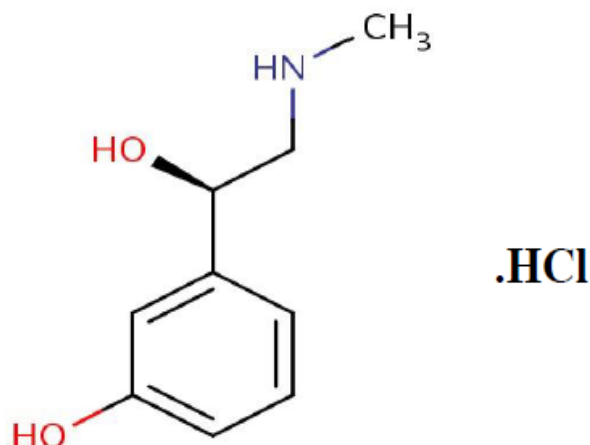
**Fig 2:** Chemical Structure of Phenylephrine HCl

This combination is used for the temporary relief of redness, burning, irritation and discomfort due to dryness of the eye or due to exposure to wind, dust, sun, computer etc [5].

Analytical methods for both drugs are reported for their combination with other drugs of same class. Literature survey revels that analytical methods like UV Spectrophotometry and HPLC [6-13]. However, there is requirement for better method for simultaneous estimation of both drugs for quality control. The reported method is very simple, quick and economical.

**2. Materials and methods**
**2.1 Instruments**
Waters 2487 HPLC system having Waters 515 pumps and dual wavelength absorbance detector, connected to a computer loaded with Empower 2 for all the Chromatographic measurements.

**2.2 Reagents and Chemicals**
Pure drug sample of Naphazoline HCl was kindly gifted by Divine Laboratories, Padara and pure drug sample of Phenylephrine HCl was kindly gifted by Divi's Laboratory, Andhra Pradesh. The gift samples were used as standard without further purification. HPLC grade water and acetonitrile was used to prepare mobile phase and procured from Rankem Ltd., Mumbai. Potassium dihydrogen orthophosphate o-Phosphotic acid and triethylamine were of analytical grade and procured from Rankem Ltd., Mumbai. Commercial eye drops was purchased from local market.

**2.3 Preparation of Mobile Phase:** Dissolved 2.4 gm of KH2PO4 in 800 ml of HPLC grade water and add 0.1 ml of Triethylamine. Adjust the pH 3 with o-Phosphoric acid. Add 200 ml HPLC grade Acetonitrile to make ratio Phosphate Buffer: Acetonitrile: Triethylamine (80:20:0.01v/v/v).

**2.4 Preparation of standard solutions**
**NAPH stock solution**: Accurately weighted 50 mg NAPH was taken in 50 ml volumetric flask and then diluted with mobile phase up to the mark (1000μg/ml). 5 ml of this solution was transferred in 50 ml volumetric flask and diluted up to mark with mobile phase (100μg/ml).

**NAPH working solution**: 1, 2, 3, 4, 5 and 6 ml of resultant solution was transferred in 10 ml volumetric flask and diluted up to mark with mobile phase to get concentrations of 10, 20, 30, 40, 50 and 60μg/ml respectively.

**PHE stock solution**: Accurately weighted 120 mg PHE was taken in 50 ml volumetric flask and then diluted with mobile phase up to the mark (2400μg/ml). 5 ml of this solution was transferred in 50 ml volumetric flask and diluted up to mark with mobile phase (240μg/ml).

**PHE working solution**: 1, 2, 3, 4, 5 and 6 ml of resultant solution was transferred in 10 ml volumetric flask and diluted up to mark with mobile phase to get concentrations of 24, 48, 72, 96, 120 and 144μg/ml respectively.

**2.5 Analysis of marketed formulation** Pharmaceutical formulation contains NAPH 0.05% and PHE 0.12% as clear liquid solution. 2 ml of formulation (equivalent to 1mg of NAPH and 2.4 mg of PHE) was taken in 50 ml volumetric flask and diluted up to the mark with mobile phase to get NAPH 20μg/ml and PHE 48μg/ml.

**2.6 Method optimization**
To produce an optimized method for simulteneous estimation of both drugs various trials were carried out by varying solvents, wavelength, flow rate and stationary phase. Analytical wavelength was selected by scanning 20μg/ml NAPH and 48μg/ml PHE solution in the UV range of 200nm to 400nm using mobile phase as blank. 276nm wavelength was found suitable for both drugs. Grace C18, (250 mm X 4.6 mm i.d., 5 μm particle size) column. Was used as stationary phase. Mobile phase consisted of Potassium Dihydrogen Phosphate buffer pH 3: Acetonitrile: Triethyleamine in the ratio of 80:20:0.01 v/v/v (pH 3 adjusted with o-Phosphoric acid) shows good separation at the flow rate of 1ml/min. Injection volume was 20μl and ambient temperature found suitable in present method. Optimized conditions are shown in table 1.

**Table 1:** Optimized chromatographic conditions for proposed method

| Parameter | Chromatographic conditions |
|---|---|
| Instrument | Waters 2847 |
| Column | ODS C-18 Grace (250mm × 4.6mm, 5 μm) |
| Mobile phase | Phosphate buffer pH 3: Acetonitrile: Triethyleamine (80:20:0.01 v/v/v) |
| Diluent | Mobile Phase |
| Flow rate | 1ml/min |
| Wavelength | 276 nm |
| Runtime | 10 min |
| Temperature | Ambient |
| Injection Volume | 20μl |

**3. Method Validation**
The developed method of analysis was validated as per ICH Q2 (R1) [14] guidelines for parameters like system suitability, linearity, pracision accuracy, robustness, limit of detection (LOD) and limit of quantitation (LOQ).

**3.1 System suitability**
System suitability test is important part of analytical method. It ascertains the effectiveness and capability of analyzing the drugs in pharmaceutical dosage form. Initially column was saturated with mobile phase for half an hour and then blank solution was injected followed by six replicate injections of standard solution to ascertain the system suitability. The parameters such as retention time, theoretical plates, tailing factor and resolution are shown in table 2.

**Table 2:** System suitability parameters

| Parameter | Ideal condition | PHE | NAPH |
|---|---|---|---|
| Retention time | Above 1.5 min | 3.154 | 5.976 |
| Theoretical plates | NLT 2000 | 4050 | 3100 |
| Tailing Factor | NMT 2.0 | 1.3 | 1.4 |
| Resolution | NLT 2.0 | 9.3 | |
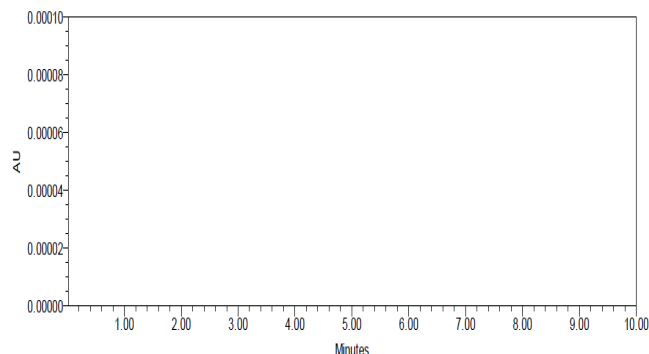
**Fig 3:** Chromatogram of Blank
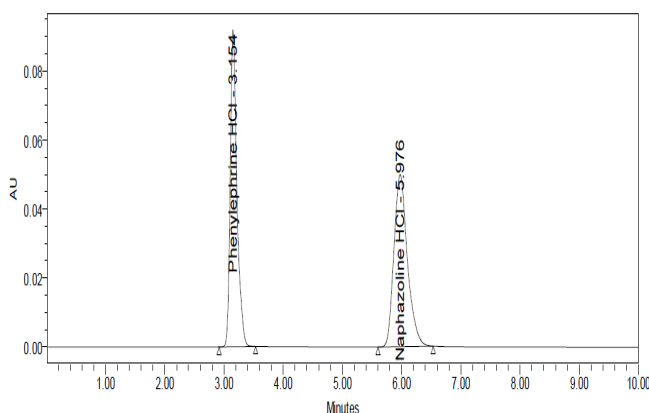
**Fig 4:** Chromatogram of NAPH and PHE

### 3.2 Linearity

The proposed RP-HPLC method was applied in the concentration range of 10-60μg/ml for NAPH and 24–144μg/ml for PHE. Calibration curve of peak area Vs concentration was plotted. The correlation co-efficient and regression equation were determined. Chromatograms over linearity range for both drugs are shoen in fig 5 and 6. The calibration curves and regression data are shown in fig 7 and 8 for NAPH and PHE respectively.
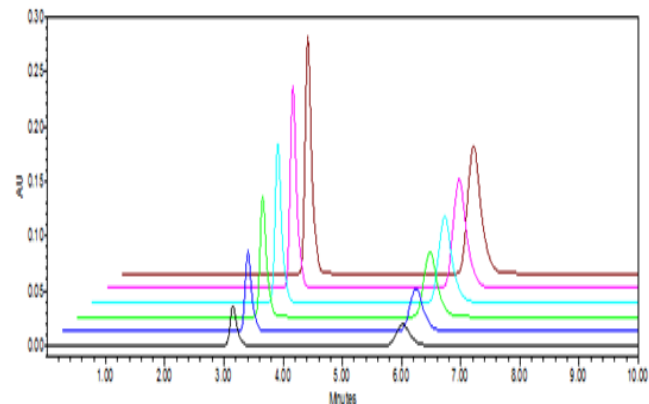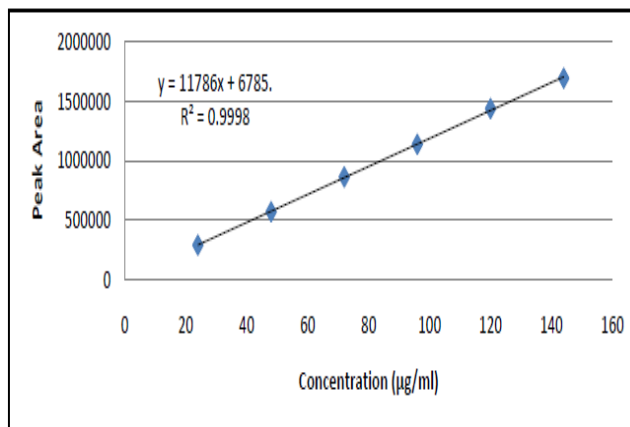
**Fig 5:** Overlain Chromatogram of NAPH and PHE (3D)
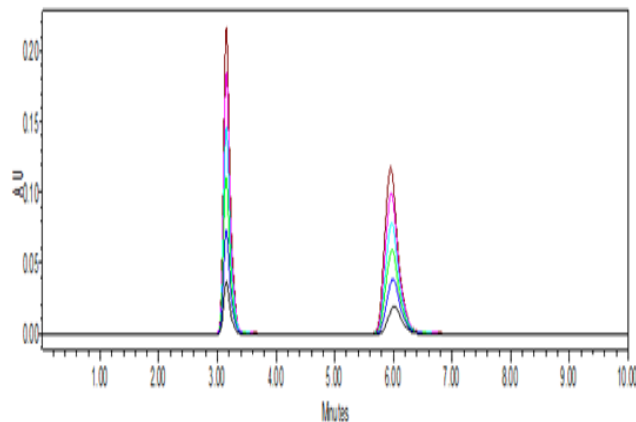
**Fig 6:** Overlain Chromatogram of NAPH and PHE (2D)
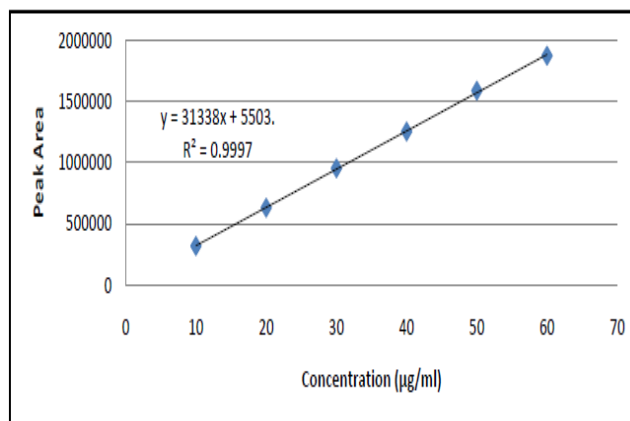
**Fig 7:** Calibration curve of NAPH

$y = 31338x + 5503.$
$R^2 = 0.9997$

**Fig 8:** Calibration curve of PHE

$y = 11786x + 6785.$
$R^2 = 0.9998$

### 3.3 Precision

For repeatability, six replicates of standard mixture solution having NAPH (30μg/ml) and PHE (72μg/ml) were prepared and peak area were recorded and RSD was calculated. Intraday and Interday precision for proposed method were measured in terms of % RSD. The experiment was repeated three times in a day for intraday and on three different days for interday precision. The limit for %RSD is NMT 2%.

### 3.4 Accuracy (Recovery Study)

Accuracy was determined by performing recovery studies by spiking different concentrations of pure drug in pre analyzed

sample solution of 20μg/ml of NAPH and 48μg/ml of PHE. To pre-analyzed sample solution, a known amount of standard stock solutions were added which was at different level 80, 100 and 120%. The solutions were analyzed by proposed method. Mean % recovery was calculated.

**3.5 LOD and LOQ:** Calibration curves were repeated 6 times and standard deviation of intercept were calculated. Then LOD and LOQ were measured as follows.
LOD = 3.3 * SD/ Slope of calibration curve
LOQ = 10 * SD/ Slope of calibration curve
SD = Standard deviation of intercepts.

**3.6 Robustness:** The robustness of the method was determined by changing flow rate of mobile phase, by changing the detection wavelength and changing organic content of the mobile phase for standard drugs. Each factor selected was changed at three levels with respect to optimized parameters. Robustness of the method was carried out at 25μg/ml for NAPH and 60μg/ml for PHE.

**3.7 Applicability of method:** Applicability of proposed method was tested by analyzing marketed formulation. Acceptance limit for assay of eye drop formulation is within limit with low SD justified the assay of method.

**Table 3:** Summary of Validation Parameters

| 1. Validation Parameters | | |
|---|---|---|
| **Parameters** | **Results** | |
| | **NAPH** | **PHE** |
| Precision | | |
| Repeatability | 0.35 | 0.37 |
| Intraday | 0.34 – 0.90 | 0.21 – 0.67 |
| Interday | 0.56 – 1.30 | 0.37 – 1.29 |
| Accuracy | 99.13-100.70% | 98.35-100.50% |
| LOD (μg/ml) | 0.28 | 0.48 |
| LOQ (μg/ml) | 0.85 | 1.47 |
| 2. Assay of Commercial Dosage Form | | |
| Formulation | Assay found (% found ± SD) (n=6) | |
| Eye Drops | 99.80% ± 0.85 | 99.78% ± 0.58 |

## 4. Results
An RP-HPLC mathod for estimation of Naphazoline HCl and Phanylephrine HCl having mobile phase Phosphate buffer pH 3: Acetonitrile: Triethyleamine in ratio of 80:20:0.01 v/v/v shows good saperation at flow rate of 1ml/min with ODS C-18 Grace (250mm × 4.6mm, 5 μm) as stationary phase at ambient temperature. The calibration curve was linear in concentration ranges of 10-60μg/ml for NAPH and 24-144μg/ml for PHE. Accuracy was determined by calculating the recovery, and the mean. Precision was calculated as repeatability (relative standard deviation) and intra and inter day variation (%RSD) for both drugs. The %RSD values for both drugs were found to be less than 2, which indicate that the method is precise. LOD values for NAPH and PHE were found to be 0.28 & 0.48μg/ml respectively. LOQ values for NAPH and PHE were found to be 0.85 & 1.47μg/ml respectively indicates sensitivity of the proposed method.
The method was successfully used to determine the amount of NAPH and PHE in pharmaceutical formulation. The results obtained are in agreement with the corresponding labelled amount. By observing the validation parameters, the methods were found to be precise, accurate and sensitive. Hence these methods can be used for routine analysis.

## 5. Discussion
In RP HPLC method, the primary requirement for developing a method for analysis that the using different solvents and buffers and columns to get better retention time and theoretical plates and better cost effective and time saving method than the previously developed methods. The proposed method allows ease of analysis and reproducibility. The proposed method is robust and useful for analysis of pharmaceutical dosage form accurately and quick.

## 6. Conclusions
RP-HPLC method was developed for simultaneous estimation of Naphazoline HCl and Phenylephrine HCl in pharmaceutical dosage form. The proposed method was found to be simple, accurate, and precise and have ability to detect the drug from excipients in the market formulation. The method was validated as per ICH Guidelines Q2 (R1). The method is suitable for routine Quality analysis of NAPH and PHE in the pharmaceutical dosage form.

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