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DEVELOPMENT AND VALIDATION OF A HPLC-UV METHOD FOR SIMULTANEOUS DETERMINATION OF PHENYLEPHRINE HYDROCHLORIDE AND KETOROLAC TROMETHAMINE IN OCULAR FORMULATION

Naveen Kumar G S[*1] and Srinivasa U [2]

[1]Ph.D Research scholar Pacific Academy of Higher Education and Research University Udaipur, Rajasthan, INDIA and Asst Professor, Department of Pharmaceutical Analysis, Sarada Vilas College of pharmacy, Mysuru, Karnataka, India,

[2]HOD Department of Pharmacognosy, Srinivas College of pharmacy, Mangalore, Karnataka India,

Abstract:

A simple, rapid, and sensitive high-performance liquid chromatographic method with UV detection has been developed and validated according to the ICH guidelines for the quantitation of phenylephrine Hydrochloride (PHH), Ketorolac Tromethamine (KET) in pharmaceutical dosage form. Chromatographic separation were achieved on C18 column (250 mm × 4.6 mm; 5 µm particle size) with simple mobile phase composition of 10 mM Potassium dihydrogen phosphate buffer with Triethylamine (pH 3.14) and acetonitrile (40:60, v/v) at a flow rate of 0.5 mL min-1 where detector was set at 302 nm with a total run time of 8 mins. The method was linear over the concentration range of 40-100, µg mL-1 with a correlation coefficient of 0.9891 and 0.994. Limit of quantifications (LOQ) of 13.3, 26.3 and limit of detections (LOD) 4.4, 8.7 µg mL-1 for PHH, and KET respectively. Accuracy and precision values of both within-run and between-run obtained from six different sets of three quality control (QC) samples analyzed in separate occasions for both the analytes ranged from 98.15% to 99.85% and 0.95% to 2.13%, respectively. Extraction recovery of analytes in pharmaceutical formulation from 97.72% to 99.78%. The developed and validated method was successfully applied to quantitative determination of PHH and KET in pharmaceutical formulation.

Keywords: *High performance liquid chromatography, UV, Spectrophptometry, Phenylephrinehydrochloride & Ketorolac Tromethamine.*

Corresponding author:

Naveen Kumar G.S,
Ph.D Research scholar,
Pacific Academy of Higher Education and Research University Udaipur,
Rajasthan, INDIA
Email: naveengs.2010@gmail.com

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INTRODUCTION:

Phenylephrine hydrochloride (PHH) is a selective α_1-adrenergic receptor agonist of the phenethylamine class used as a decongestant, as an agent to dilate the pupil, and to increase blood pressure figure 1
derivative figure 2, a nonsteroidal antiinflammatory drug, is indicated for short-term management of moderate to severe pain and shows a high incidence of side effects like gastric bleeding[2]. The primary mechanism of action responsible for ketorolac's anti-inflammatory, antipyretic and analgesic effects is the inhibition of prostaglandin synthesis by competitive blocking of the enzyme cyclooxygenase (COX).

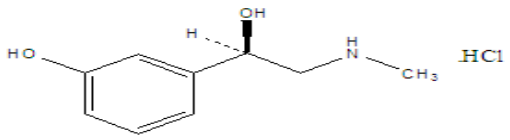


Fig 1: Phenylephrine hydrochloride

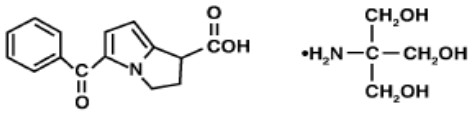


Fig.2:Ketorolac trome

Literature survey revealed various methods for determination of Ketorolac tromethamine and PhenylephrineHydrochloride in their combined form[5-13].An extensive review of the literature did not revealed any HPLC method for simultaneous determination of both drugs. Therefore, attempts were made to develop and validate simple, precise, and sensitive, isocratic reverse phase stability indicating high performance liquid chromatographic method for simultaneous determination of both drugs in pharmaceutical formulations.The proposed methods were validated in compliance with the ICH guidelines and were successfully applied for determination ofPhenylephrine Hydrochloride and Ketorolac Tromethamine in their pharmaceutical formulations.

MATERIALS AND METHODS:

Instrument

HPLC- LC100 UV– Detector, Model LC-100 Systronics.

Reagents and Chemicals

PHH, KET were procured from pharmaceuticals industry. Acetonitrile HPLC Grade, Phosphoric acid analytical grade from Merck (Mumbai, India), Potassium dihydrogen phosphate analytical grade from Merck (Mumbai, India), Triethylamine analytical grade from Merck (Mumbai, India), 1[1].Ketorolac tromethamine (KET) (±)-5-benzoyl-2,3dihydro-1H-pyrrolizine-1-carboxylic acid, a compound with 2-amino-2-(hydroxymethyl)-1,3propanediol, a pyrrolizine carboxylic acid HPLC-grade water (resistivity 18.2 MΩ) cm was generated from a Milli-Q water purification system, was used throughout the analysis. Samples are procured from pharmaceutical industry and they are considered as Sample I and Sample II respectively and both the samples are ocular formulations.

Instrumentation and chromatographic conditions

Chromatographic separation was achieved by using systronics LC-100 high-performance liquid chromatography, equipped with degasser PGU-20A 5, variable wavelength programmable diode array detector UV, auto sampler SIL-20 AC HT, and column oven CTO-10 A5 VP. Pronto SILC 18, 250 × 4.6mm ID, 5 μm particle size was used as the stationary phase. The column temperature was kept at 37^0C, and the mobile phase flow rate was maintained at 0.5mL min−1.

Mobile phase consisted of a mixture of Potassium dihydrogen phosphate buffer (2.7363 gm to 1000ml+ 1 ml Triethylamine, pH 3.14 by dil H_3PO_4) and Acetonitrile (40:60, v/v) at a flow rate of 0.5 ml min-1 and sample injection of 20 µL was injected at 25oC. Eluent was monitored with a UV detector set at 302 nm.

Preparation of stock and working solutions

24.5 gm of PHH and 25.5 gm of KET taken in a 25 ml volumetric flask and dissolving in mobile phase to obtain concentration of 1000 µg/mL. The stock solution stored in amber colored labeled volumetric flask at 8 ^{0}C.

Preparation of calibration standards and quality control (QC) samples

Five calibration standards (CC) of both PHH and KET at concentration of: 20, 40, 60, 80, and 100 µg mL-1 were prepared by spiking 0.2, 0.4, 0.6, 0.8, 1.0 ml respectively to 10 ml by Mobile phase. Three QC sample 40, 60, 80 µg mL-1 were used. All standards stored in amber colored labeled volumetric flask at 8 ^{0}C.

Sample preparation

2ml of sample diluted with mobile phase mixed properly. Samples were further diluted by mobile phase which have final concentration 20µg mL-1 of both PHH and KET and then injected into the HPLC system.

Method validation

The proposed methods were validated in compliance with the ICH guidelines and were successfully applied for determination of Phenylephrine Hydrochloride and Ketorolac Tromethamine in their pharmaceutical formulations. This method was validated to meet the acceptance criteria with the ICH guidelines of method validation[4].

Selectivity

Selectivity of the method was determined by analyzing blank (mobile phase), to demonstrate the lack of chromatographic interference at the retention time of the analytes.

Limit of detection (LOD), Limit of quantitation (LOQ) and Linearity

Limit of detection (LOD), Limit of quantitation (LOQ) was determined by the following equation 3.3xσ/S and 10xσ/S, whereas σ = standard deviation of the response and S = slope of the calibration curve. Calibration curves were acquired by plotting the peak-area of the analytes against the nominal concentration of calibration standards. Analyte concentration of different CC and QC samples were prepared as mentioned above.

Accuracy and precision

Accuracy of an analytical procedure is the closeness of agreement between accepted conventional true values (reference values) and the values found. The accuracy of the proposed methods was tested by the determination of PHH and KET at different concentration levels within the linear range of each compound. Precision was studied by determination of intra-day and inter-day precision. Intra-day precision was determined by injecting five standard solutions of three different concentrations on the same day and inter-day precision was determined by injecting the same solutions for three consecutive days. Relative standard deviation (RSD %) of the peak area was then calculated to represent precision.

Extraction recovery

Recoveries of PHH and KET were determined in the addition standard (40, 60, 80 µg mL-1) by
Comparing the experimental and true values (40, 60, 80 µg mL-1).

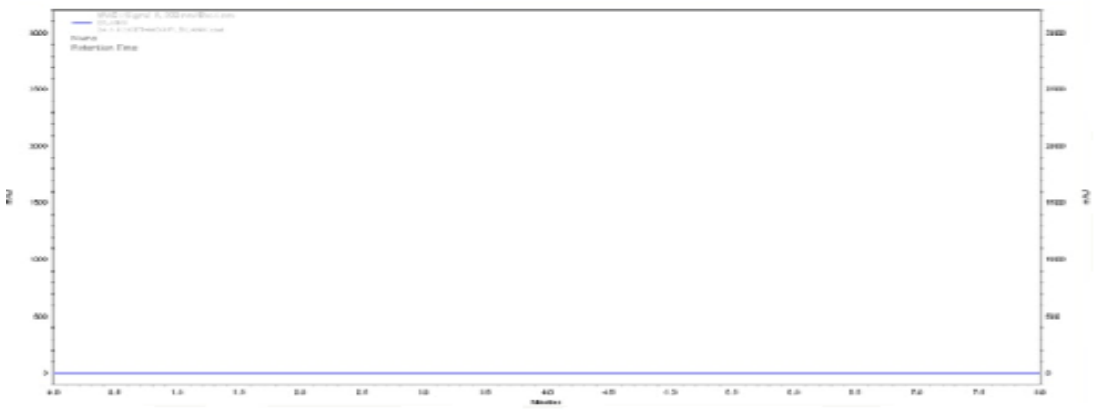
RESULTS AND DISCUSSION:

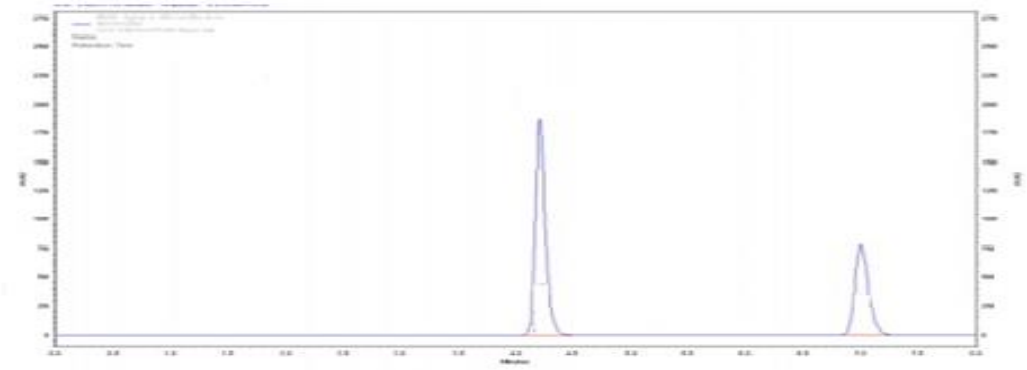
Optimization of chromatography

In the work chromatographic conditions were optimized to obtain high sensitivity, reproducibility, and sample throughput for the determination of PHH and KET. Both analytes contain basic nitrogen atoms and therefore have the potential to cause the peak tailing due to interaction of this basic nitrogen atoms with the silanol group of the stationary phase during chromatographic separation. In order to obtain symmetrical peaks with better resolution, the chromatographic condition i.e. pH of the buffer, concentration of the organic modifier and silanol blocker were optimized. Various chromatographic condition such as mobile phase composition, analytical column with different packing materials (C18,Phenyl, Cyano) and configuration (10, 15, 25 cm) were used to obtain sharp peak with reduce tailing, and better resolution with no peak impurity. Finally Agilent Zorbax Eclipse Plus C18 column was selected which provided reduced peak tailing and acceptable peak purity index. Eclipse plus C18 packing is made by first chemically bonding a dense monolayer of dimethyl-n-octadecylsilane stationary phase to a specially prepared, an improved ultra-high purity (>99.995% SiO2), ZORBAX Rx-SIL porous silica support. The bonded-phase packing is doubly endcapped using proprietary reagents and procedures to obtain maximum deactivation of the silica surface. Mobile phase composition was selected based upon the peak parameter (symmetry, tailing, resolution and peak purity index etc.), run time, case of preparation and cost. The most suitable mobile phase composition was found to be acetonitrile and phosphate buffer (pH 3.14) in the ratio of 60:40 (v/v), respectively. Under the chromatographic conditions outline, highly symmetrical, sharp peaks of PHH and KET were obtain at retention time about 4.2 and 7.0 min. respectively.

Selectivity

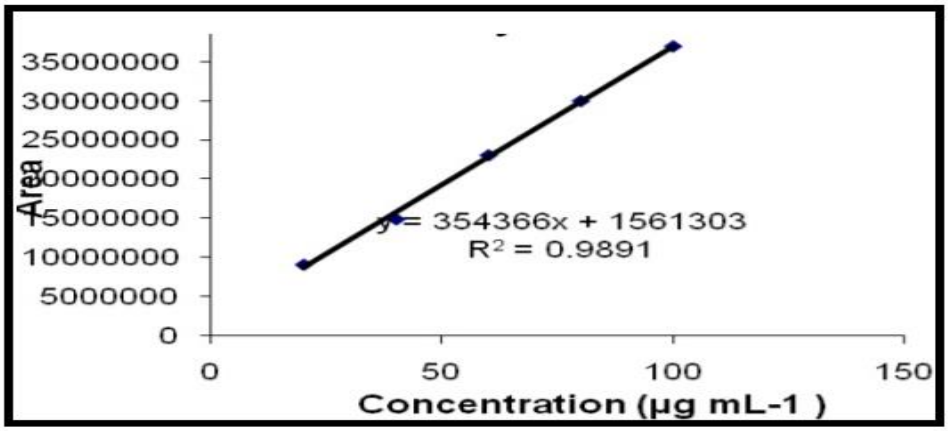
The method was found to selective as no significant interfering peak is observed at the retention times of PHH and KET which were 4.2, and 7.0 minrespectively. Total chromatographic run time was 10.0 min. (Figure 3 & 4) shows the representative chromatograms of blank spiked with analytes .

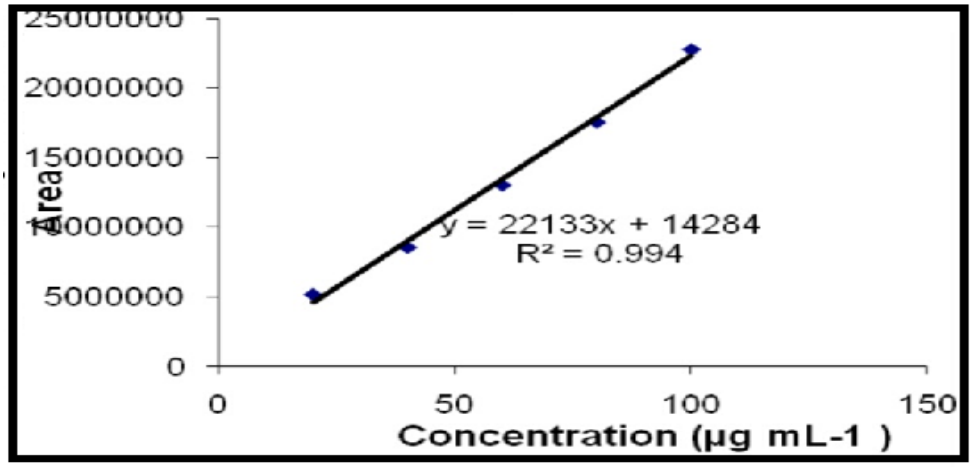




Limit of detection (LOD), Limit of quantitation (LOQ) and Linearity

Limit of detection (LOD) was established 4.4 and 8.7 µg mL-1 for PHH and KET respectively. Limit of quantification (LOQ) was established 13.5 and 24.3 µg mL-1 for MOX and KET respectively. Calibration curves were linear over the concentration range 40–100 µg mL-1 of both analytes. Regression coefficient 0.9893 and 0.996 for PHH and KET respectively. (Figure 5 & 6) Standard curve had a reliable reproducibility over the standard concentrations across the calibration range. All back-calculated concentrations did not differ from the theoretical value as no single calibration standard point was dropped during the validation.





Accuracy and precision

The accuracy and precision of the proposed methods were tested by the determination of PHH and KET at different concentration levels within the linear range of each compound. The low SD (< 1) of six determinations indicated the high accuracy and precision of the proposed method. Collective results are shown in Tables 1 & 2. The inter- and intra-day determination of PHH and KET over 3 consecutive days by the same analyst using the same instrument is shown in Tables 1 & 2. The low RSD (< 2%) reflects the ruggedness of the methods.

Table 1: Assessment of Accuracy and Precision of Ketorolac Tromethamine

	QC Sample (µg mL-1)	Mean (µg mL-1)	SD	RSD%	Accuracy %
Intra Day (N=6)	40.00 60.00 80.00	38.72 59.54 79.34	0.71 1.29 1.27	1.78 2.14 1.59	99.29 99.18 99.24
Inter Day (n=18)	40.00 60.00 80.00	39.58 59.54 79.25	0.72 1.19 1.37	1.79 1.96 1.71	98.88 99.07 99.06

S.D. = Standard deviation; R.S.D. (%) (Relative standard deviation) = [(S.D./Mean) X 100]; Accuracy (%) = [(Mean / Conc. Added) X 100]; n = number of replicates.

Table 2: Assessment of Accuracy and Precision of Phenylephrine Hydrochloride

	QC Sample (µg mL-1)	Mean (µg mL-1)	SD	RSD%	Accuracy %
Intra Day (N=6)	40.00 60.00 80.00	39.82 59.28 79.36	0.57 0.79 1.27	1.43 1.32 1.59	99.83 98.80 99.24
Inter Day (n=18)	40.00 60.00 80.00	39.48 58.92 78.81	0.71 0.76 1.02	1.81 1.28 1.29	98.68 98.17 98.49

S.D. = Standard deviation; R.S.D. (%) (Relative standard deviation) = [(S.D./Mean) X 100];
Accuracy (%) = [(Mean / Conc. Added) X 100]; n = number of replicates.

Table 3: Extraction Recovery of Analyst n=6

Analyte	QC sample (µg mL-1)	Extraction Recover (%)	RSD (%)
KET	40.00	98.05	0.90
	60.00	98.24	0.84
	80.00	98.10	0.70
PHH	40.00	98.65	0.71
	60.00	98.59	1.04
	80.00	97.90	1.09

R.S.D. (%) (Relative standard deviation) = [(Standard deviation /Mean) X 100]; n = number of replicates.

Table 4: Estimation Ketorolac and Phenylephrinein Different Formulation

Sample	Analyte	Concentration found % (w/v)	%
Sample I	KET	0.255	101.98
	PHH	0.245	102.92
Sample II	KET	0.259	98.76
	PHH	0.247	98.84

Extraction recovery

Recovery results were found to be satisfactory as these were consistent, precise and reproducible are summarized in **Table 3.**

Implementation to Pharmaceutical formulation:

This newly developed method was applied to determine the KET and PHH in pharmaceutical formulation (eye drops). Result ware summarized in **Table 4.**

CONCLUSION:

Here, we have developed and validated a HPLC-UV method that has significant advantages over the previously published method as it provides simple mobile phase composition for chromatographic separation, shorter run time for analysis, simple sample preparation as well as improved sensitivity. Therefore, this new method leads to a simple, feasible, cost effective, rapid method with high degree of accuracy and specificity to quantify simultaneously PHH and KET in pharmaceutical formulations with HPLC-UV. It will be extremely helpful for successfully analyzing the PHH and KET in ocular formulations.

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