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A VALIDATED RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF PHENYLEPHRINE AND KETOROLAC IN INJECTABLE PREPARATIONS

P. J. Prasuna Sundari[1]*, Anitha kusuma[2], Prathima Srinivas[3]

Department of Pharmaceutical Analysis and Quality Assurance, Sri Venkateshwara College of Pharmacy, Osmania University, Hyderabad -500081, India.

***Corresponding Author:** *Email:* jpspingali2014@gmail.com

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ABSTRACT

Omidria is an ophthalmic injectable of phenylephrine and ketorolac intended for use in cataract surgeries and intraocular lens replacement procedures. Objective of the present study was to develop and validate a specific, accurate, precise RP-HPLC method for the simultaneous estimation of phenylephrine and ketorolac in omidria. The proposed RP- HPLC method utilizes Std BDS C8column (250 mm × 4.6 mm id, 5µm particle size), mobile phase consisting of buffer and acetonitrile in the ratio of (30:70) and a flow rate of 1 ml/min. Quantitation was achieved with PDA detection at 220 nm. Separation was achieved at retention times of 2.313min and 3.090min for phenylephrine and ketorolac respectively. The method was validated as per ICH guidelines for accuracy, precision, linearity, limit of detection, limit of Quantitation and robustness. Limit of detection and limit of Quantitation were determined to be 0.091ppm 0.087ppm; 0.278ppm 0.021ppm for phenylephrine and ketorolac respectively. Linearity was achieved at concentration range of 20-120µg/ml and 6-36µg/ml for phenylephrine and ketorolac. It can be concluded from this study, that the developed RP-HPLC method may be applied in regular analysis for simultaneous estimation of phenylephrine and ketorolac in various dosage formulations.

Keywords **–** Omidria, Phenylephrine, Ketorolac, RP-HPLC, Validation.

1. INTRODUCTION

"Omidria" is an ophthalmic injectable of Omeros Corporation, USA and as per the label claim 4ml of each vial contains phenylephrine 40mg, ketorolac 12mg. The product is intended for use in ophthalmic procedures such as cataract surgery or intraocular lens replacement (ILR). Its use is to maintain pupil size by preventing intraoperative miosis (pupil constriction) and to reduce postoperative pain. Ketorolac is a non-steroidal anti-inflammatory drug (NSAID) and is used principally for its analgesic activity.

1.1 Phenyleprine

Phenylephrine is a sympathomimetic amine that acts predominantly on α-adrenergic receptors. It is mainly used to treat nasal congestion and also useful in treating hypotension and shock.[1]

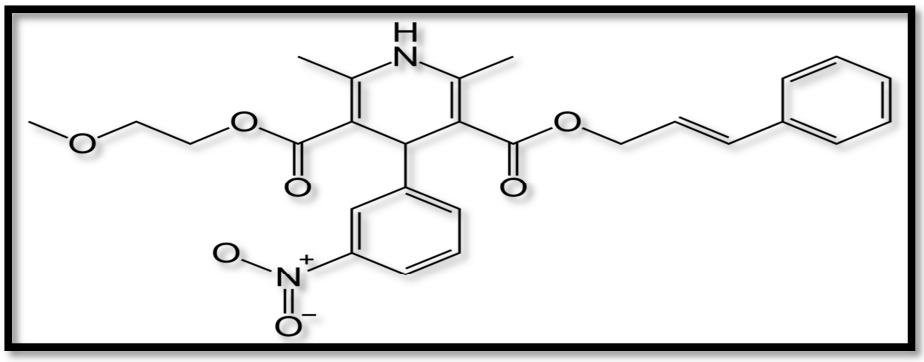

Fig.1: Chemical structure of Phenylephrine

1.1.1 Physicochemical data

IUPAC NAME	: 3-[(1R)-1-hydroxy 2- (methyl amino) ethyl] phenol
Chemical Formula	: $C_9H_{13}NO_3$
Mol. Weight	: 167.205
Characteristics	: Crystalline powder
Physical State	: solid
Solubility	: Soluble in water, DMSO, methanol.
Storage	: Store at room temperature
Melting Point	:143-145 °C
pK_a	: 8.86

Literature survey reported the use of several analytical methods for the determination of phenylephrine. Wichran Janwitayanuchit[2]et al (2014) developed a HPTLC method for the determination of Brompheniramin maleate and Phenylephrine hydrochloride in tablet dosage form. Rushikesh Bandelwar[3] et al (2013) have developed an RP-HPLC method for estimation of Phenylephrine hydrochloride, Chlorpheniramine maleate, Paracetamol and Caffeine in bulk drug and tablet dosage form. Palled Mahesh[4] et al (2013) developed an RP-HPLC method for determination of Acetaminophen, Caffeine and Phenylephrine hydrochloride and Dextromethorphan hydro bromide in tablet dosage form. Pinak M[5] et al (2013) developed a validated RP-HPLC method for estimation of Chlorpheniramine maleate, Ibuprofen and Phenylephrine hydrochloride in combined pharmaceutical dosage form. Nora H[6] et al (2010) developed HPLC and UV methods for determination of Phenylephrine and Chlorpheniramine in binary mixture.

1.2 Ketorolac

A pyrrolizine carboxylic acid derivative structurally related to indomethacin. It is a non steroidal anti inflammatory drug (NSAID) and is used principally for its analgesic activity. Its anti inflammatory effects are due to inhibition of both cylooxygenase-1 (COX-1) and cylooxygenase-2 (COX-2) thereby causing inhibition of prostaglandin synthesis[8].

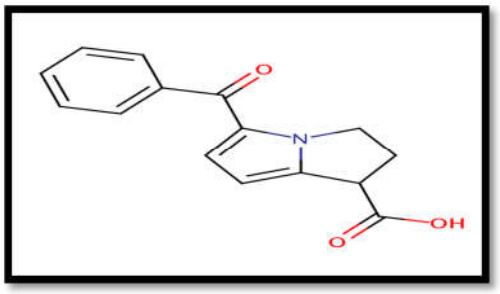

Fig.2: Chemical structure of Ketorolac

1.2.1 Physicochemical data

IUPAC Name : 5-benzoyl-2, 3-dihydro-1H-pyrrolizine-1-carboxylic acid

Chemical Formula : $C_{15}H_{13}NO_3$

pKa : 3.5

Category : Anti-inflammatory agents,
Non-steroidal cyclo oxygenase inhibitors.

Literature survey revealed that several analytical methods reported for the determination of ketorolac. B. Raja[9] et al (2014) developed a validated RP-HPLC method for simultaneous estimation of Febuxostat and Ketorolac in tablet dosage form. B.Prathap[10] et al (2011) developed an RP-HPLC method for simultaneous estimation of Febuxostat and Ketorolac in bulk and pharmaceutical dosage form in rat plasma. S.K Dubey[11] et al (2013) developed a rapid and sensitive RP-HPLC method for estimation of Ketorolac in pharmaceuticals using weighted regression. M.Jambulingam[12] et al (2013) developed a validated RP-HPLC method for Ketorolac Tromethamine in eye drop formulation. Vandana Patil[13]et al (2013) developed HPTLC method for the simultaneous analysis of Gatifloxacin and Ketorolac Tromethamine in eye drops. Boyka G[14] et al (2013) developed HPLC method for determination of Ketorolac Tromethamine in tablet dosage forms. Irfana Mariam[15] et al (2012) developed a stability indicating HPLC method for the simultaneous determination of Ofloxacin and Ketorolac Tromethamine in pharmaceutical formulations.

The present study aimed to develop a new simple, precise and accurate RP-HPLC method for the simultaneous estimation of phenylephrine and ketorolac and validate the method according to ICH guidelines.

2. MATERIALS AND METHODS

2.1 Instrument

Analysis was performed on HPLC Waters 2695 with 2996 module having photo diode array detector, equipped with a quaternary solvent delivery pump, automatic sample injector and column thermostat. The data acquisition and analysis was performed by using Empower software.

2.2 Chemicals and reagents

Reference standard of phenylephrine and ketorolac were gifted by Spectrum laboratories Kukatpally. Omidria was procured from the local pharmacy. Methanol, acetonitrile, ortho phosphoric acid, milli-Q water all were obtained from Merck chemicals Mumbai and all other solvents used were of analytical grade.

2.3 Preparation of solutions

2.3.1 Buffer: (0.1%OPA) 1ml of orthophosphoric acid solution was transferred into a 1000ml volumetric flask containing 100ml of Milli-Q water, stirred and was made up to volume with Milli-Q water.

2.3.2 Preparation of mobile phase

Mobile phase: Measured volumes of 30ml of buffer and 70ml of acetonitrile were taken into a measuring cylinder. The solution was sonicated and degassed (30:70v/v).

2.3.3 Diluent: Measured volumes of 50ml each of methanol and water were taken into a measuring cylinder. This solution was sonicated and degassed (50:50v/v).

2.3.4 Preparation of working standard solution of phenylephrine

Weighed accurately 25mg of phenylephrine into 25ml clean and dry volumetric flask. Sufficient volume of the diluent was added and sonicated for 30 min and was made upto the mark with diluent (Stock solution).

0.8ml of phenylephrine stock solution was pipeted into 10ml volumetric flask, diluted up to the mark with diluent (concentration of phenylephrine 80µg/ml).

2.3.5 Preparation of working standard solution of ketorolac

Weighed accurately 7.5mg of ketorolac into 25ml clean and dry volumetric flask containing sufficient volume of the diluent. The solution was sonicated for 30min and diluted up to the mark with diluent (stock solution).

0.8ml of above stock solution was pipetted into 10ml volumetric flask and diluted up to the mark with diluent (concentration of ketorolac 24µg/ml).

2.3.6 Preparation of sample solution

The contents of a vial of Omidria (4ml) were transferred into a 25 ml volumetric flask and made upto the volume using diluents (stock solution).

0.5ml of sample stock solution was pipetted into 10 ml volumetric flask and diluted up to the mark with diluent (concentration of phenylephrine 80µg/ml and ketorolac 24µg/ml).

2.3.7 Method development trials

Chromatographic elution was carried out in isocratic mode using mobile phase consisting of buffer and acetonitrile in different ratios and the column temperature was maintained at 30°C.The analysis was performed at a flow rate of 1.0ml/min and runtime of 10min. Detection was carried out at a wavelength of 220nm.

Table 1: HPLC isocratic programming

Trial No	Mobile phase OPA buffer: Acetonitrile	Column
1	55:45	Std ODS 150
2	70:30	Std ODS 150
3	40:60	Std BDS C8 250 x 4.6mm 5µ
4	35:65	Std BDS C8 250 x 4.6mm 5µ
5	45:55	Std BDS C8 250 x 4.6mm 5µ
6	30:70	Std BDS C8 250 x 4.6mm 5µ

2.3.8 Assay

10µL of the sample solution was injected into the column and the peak area of drug was noted. Assay was expressed as % recovery. It was calculated by the following formula:

$$\%Assay = \frac{AT}{AS} \text{X} \frac{WS}{DS} \text{X} \frac{DT}{WT} \text{X} \frac{P}{100} \text{X} \frac{Avg.Wt}{LabelClaim} \text{X} 100$$

Where

AT=peak area of the test preparation

AS=peak of the standard preparation

WS=weight of working standard taken in mg

WT=weight of sample taken in mg

DS=dilution of standard solution

DT=dilution of sample solution

P=percentage purity of working standard

2.3.9 System suitability

System suitability test parameters were checked by six repeated injections of the working standard solution to check the reproducibility of the system. Retention time, peak area, number of theoretical plates (N), tailing factor and resolution were determined. The %RSD was calculated.

2.4 Method validation

The developed method was validated according to ICH guidelines.

2.4.1 Specificity

Weighed accurately 3.92mg each of citric acid monohydrate and sodium citrate dehydrate as placebo into a 25ml volumetric flask. Sufficient volume of the diluent was added and made up the final volume with diluent. From the above stock solution 0.5ml was pipetted into a 10ml volumetric flask and diluted up to the mark with diluent which is equivalent to the sample preparation**.** Specificity of the developed method was determined by injecting blank, placebo and working standard solution and sample solution.

2.4.2 Linearity

Solutions of different concentrations ranging from 20-120µg/ml for phenylephrine and 6-36 µg/ml for ketorolac were injected into BDS C8 column and chromatograms were recorded. The peak area versus concentration of drug was plotted and a linear least-square regression analysis was conducted to determine the slope, intercept and correlation coefficient (r) to demonstrate the linearity of the method.

2.4.3 Limit of Detection (LOD): The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value. Based on the standard deviation of the response and the slope, detection limit (LOD) may be expressed as

$$LOD = 3.3 \times \frac{\sigma}{S}$$

Where σ = the standard deviation of y- intercepts of regression line.

S = slope of calibration curve.

2.4.4 Limit of Quantitation (LOQ)

The Quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. Based on the standard deviation of the response and the slope,

The Quantitation limit (LOQ) may be expressed as

$$LOQ = 10 \times \frac{\sigma}{S}$$

Where σ = the standard deviation of y- intercepts of regression line.

S = slope of calibration curve

2.4.5 Accuracy

Accuracy was performed by determining % recovery at the level of 50%, 100%, 150%, each concentration in triplicate form for phenylephrine and ketorolac standards to the pre-analysed marketed formulation and then comparing the added concentration with the found concentration. The percentage recoveries of phenylephrine and ketorolac at each level were determined.

$$\% \text{ Recovery } = \frac{\text{Amount found}}{\text{Amount added}} \text{x} 100$$

2.4.6 Precision

The precision was determined by six repeated injections of working standard solution. The chromatograms were evaluated for retention time, peak area for both phenylephrine and ketorolac and %RSD calculated.

2.4.7 Robustness

The robustness of the method was determined by inducing deliberate changes in flow rate and column temperature. 10µl of working standard solution was chromatographed at flow rates of 0.8 and 1.2 ml/ min and at column temperature of 25ºC and 35°C. Changes in retention times and plat count were determined.

3. RESULTS AND DISCUSSION

This study attempted to develop and validate an RP-HPLC method for simultaneous estimation of phenylephrine and ketorolac in omidria. The optimized method achieved good separation of phenylephrine and ketorolac with Std BDS C8 250 x 4.6mm 5µ column and OPA buffer and acetonitrile as mobile phase in the ratio of 30:70. The injection volume was 10µl with run time of 10min which provided a good separation of phenylephrine and ketorolac with suitable peak symmetry by isocratic elution. Specificity data demonstrated that the excipients used in the formulation did not interfere with analyte peak. So the developed method is specific. The results of recovery studies are depicted in Table 6. Good recovery of the spiked drug was obtained at each added concentration. %Recovery of the phenylephrine and ketorolac were found to be 99-101% and 101.11 to 101.9% respectively. Calibration curve of peak area against concentration was found to be linear in the concentration range of 20-120µg/mL for phenylephrine and 6-36µg/mL for ketorolac. The linearity data is depicted in Table 7. Correlation coefficient of 0.9992 and 0.9994 for phenylephrine and ketorolac respectively. The minimum detectable limit was found to be 0.09ppm and 0.087ppm respectively. The minimum Quantitation limit was found to be 0.218ppm and 0.021ppm respectively. The data for precision was depicted in Table 8 and 9. The % RSD value was less than 2. The % RSD was within the acceptable limits. So the method was precise. The data of robustness study have been indicated in Table 10. It was found that there was no drastic change in the resolution of phenylephrine and ketorolac when deliberate changes were introduced in the optimized chromatographic conditions thus confirming robustness of the method.

Table 2: Method development trials for simultaneous estimation of phenylephrine and ketorolac in Omidria

Trial No	Phenylephrine Retention time	Ketorolac Retention time	Observation
1	1.752min	-	Only phenylephrine peak was eluted
2	1.785min	-	Only phenylephrine peak was eluted and retention time was below 2.
3	2.99min	5.55min	Both peaks were eluted but base line was not good.
4	2.175min	3.981min	Peaks were eluted, phenylephrine peak shows tailing and ketorolac peak shows fronting.
5	2.085min	4.410min	Two peaks were eluted but ketorolac peak shows fronting.
6	2.313min	3.090min	Two peaks showed good separation.

Method development performed by isocratic elution, using mobile phase in different ratio's and sixth trial was found to be good in terms of peak shape and resolution.

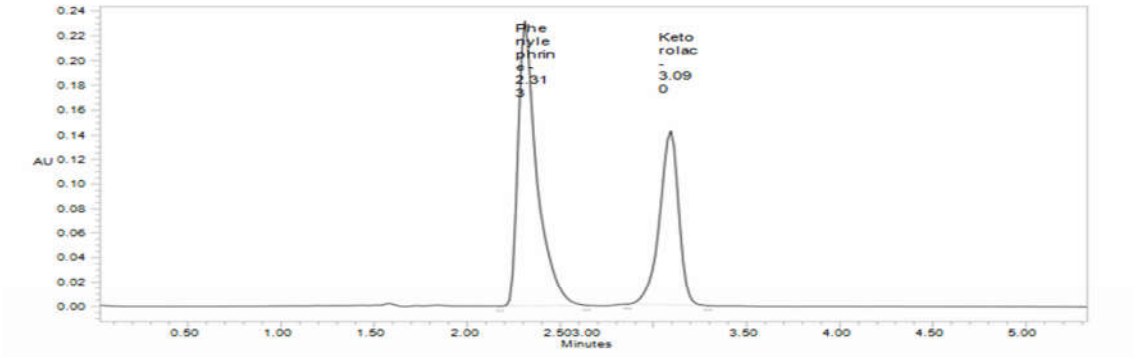

Fig.3: Trial 6 Optimized chromatogram of phenylephrine and ketorolac

Table 3: Report for Assay of phenylephrine and ketorolac

Drug	Label claim (mg)	%Purity
Phenylephrine	40	99.233
Ketorolac	12	99.01

Table 4: Results of system suitability studies

Injection no	Peak Area of phenylephrine	Peak Area of ketorolac
Injection -1	1344798	926306
Injection -2	1339312	940167
Injection -3	1349943	930313
Injection -4	1368059	937669
Injection -5	1369662	927469
Injection -6	1366036	919224
Mean	1356302	930191
SD	13212.8	7723.2
%RSD	1.00	0.80

Table 5: System suitability

Injection No	Phenylephrine			Ketorolac		
	Retention time	Theoretical plate No	Asymmetry factor	Retention time	Theoretical plate No	Asymmetry factor
1	2.122	2971	1.23	3.096	2090	1.35
2	2.131	2920	1.27	3.097	2183	1.36
3	2.135	2966	1.24	3.099	2155	1.34
4	2.151	2872	1.37	3.100	2043	1.31
5	2.163	2870	1.18	3.105	2108	1.30
6	2.169	2853	1.14	3.106	2231	1.33

Table 6: Recovery data of phenylephrine and ketorolac

Analyte	Concentration	Mean Area	Amount added	Amount found	% Recovery
Phenylephrine	50	668577	40	39.6	99
	100	1332050	80	78.92	98.65
	150	2081296	120	122.4	102
Ketorolac	50	466421	12	12.1332	101.11
	100	939796	24	24.4464	101.86
	150	1409603	36	36.684	101.9

Table 7: Results of linearity study

S. No	Concentration	Peak Area of Phenylephrine	Peak Area of Ketorolac
1	25	357487	238203
2	50	635350	495574
3	75	973624	750180
4	100	1306755	1006369
5	125	1632393	1256299
6	150	1953905	1507211

Calibration curve

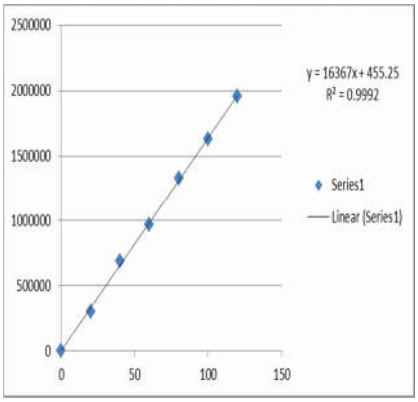

Fig. 5: Phenylephrine

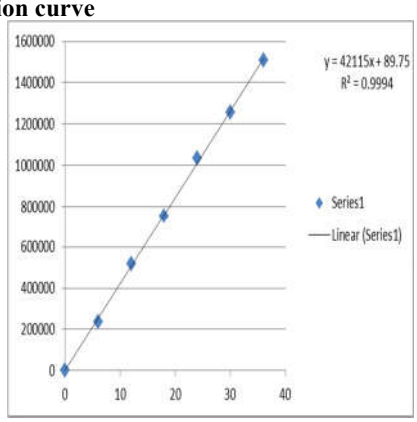

Fig. 6: Ketorolac

Table 8: Results of method precision

Injection no.	Peak area of phenylephrine	Peak area of ketorolac
1	1339883	913491
2	1351931	921932
3	1337285	929975
4	1351818	920787
5	1352429	911567
6	1351850	920994
Mean	1347533	919791
SD	6983.6	6028.7
% RSD	0.52	0.66

Table 9: Results of system precision

Injection no.	Peak area of phenylephrine	Peak area of ketorolac
1	1340242	915040
2	1327621	927616
3	1328834	923696
4	1378725	929321
5	1381092	919747
6	1336772	914679
Mean	1348881	921683
SD	24508.5	6237.4
% RSD	1.8	0.7

Table 10: Results of robustness study

Parameter	Phenylephrine		Ketorolac	
	Retention Time	Theoretical Plate No.	Retention Time	Theoretical Plate No.
Flow rate- 0.8ML	2.462	2208	3.450	2918
Flow rate- 1.2ML	2.002	2267	2.840	2855
Temperature - 25°C	2.254	2232	3.030	2845
Temperature- 35°C	2.002	2264	2.840	2849

4. CONCLUSION

A validated RP-HPLC analytical method has been developed for the simultaneous estimation of phenylephrine and ketorolac in pharmaceutical formulation. Separation was carried with BDS c8 250x 4.6mm 5µ column.The proposed method was specific, accurate, linear and precise. The developed RP-HPLC method may be applied in regular analysis for the simultaneous estimation of phenylephrine and ketorolac in all the dosage formulations.

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