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Research Article

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The estimation of Ketorolac drug in Ketorolac Tromethamine eye drops by RP-HPLC

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ABSTRACT

A simple, precise, rapid and accurate reverse phase HPLC method was developed for the estimation of Ketorolac drug in Ketorolac Tromethamine eye drops. An Alltech Spherisorb C8 150 mm x 4.6 mm, 5 µ with mobile phase consisting of Water, methanol and glacial acetic acid were mixed in the ratio 44:55:1 (v/v/v) respectively and degassed for about 10 minutes. Isocratic mode of chromatography technique was used. The flow rate was 1.2 mL/min and the eluents were monitored at 254 nm. The retention time of the Ketorolac peak is about 3 minutes. The detector response was linear in the concentration of 0.015 - 2.999 mcg/mL. The respective linear regression Y-intercept is 111.314, Residual sum of square is 310322.267 and slop is 44538.321. The limit of detection and limit of quantification were 0.0049 mcg/mL and 0.0150 mcg/mL respectively. The percentage assay of Ketorolac was 99.9 %. The method was validated by determining its accuracy, precision and linearity.

Key words: Ketorolac Tromethamine eye drops, RP-HPLC method and Isocratic mode of chromatography technique.

INTRODUCTION

Ketorolac Tromethamine is a nonsteroidal anti-inflammatory drug (NSAID) similar to ibuprofen, indomethacin, naproxen, and many others. Ketorolac blocks prostaglandin synthesis. Prostaglandins have many effects in the body including their role in pain and inflammation. In the eye prostaglandin is involved in inflammation, pain, and irritation due to allergies or mechanical injury. Ketorolac provides relief from pain and inflammation in the eyes. The FDA approved ketorolac eye drops in November 1992. Chemically, Ketorolac Tromethamine: (±)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid, 2- amino-2-(hydroxymethyl)-1,3-propanediol. The empirical formula is C19H24N2O6 with a molecular weight of 376.4 g/mol. Ketorolac Tromethamine is soluble in Water and Methanol [1-4].

Ketorolac tromethamine was not mutagenic in the Ames test, unscheduled DNA synthesis and repair and in forward mutation assays. Based on clinical use, possible target organs include the gastrointestinal system, hematopoietic system, central nervous system, cardiovascular system, liver, kidneys, and possibly the eyes. In the literature, several analytical techniques like HPLC, PIF methods including Flow Injection analysis, Spectrofluorometric, Voltammetry, GC and UV spectrophotometric methods have been reported for its determination in biological fluids and formulations. The main purpose of the present study was to establish relatively simple, sensitive and validated liquid chromatographic methods for the determination of Ketorolac in Ketorolac Tromethamine eye drops. The method was validated by determining its accuracy, precision and linearity as per ICH guidelines [5-9]

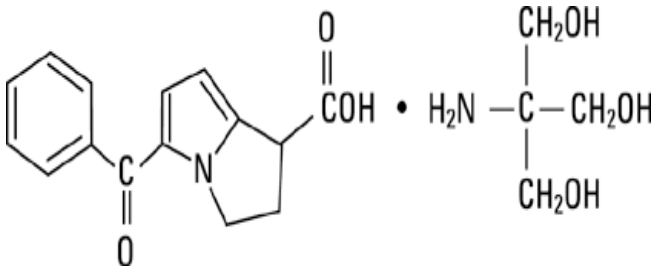


Fig 1: Structure of Ketorolac Tromethamine

EXPERIMENTAL SECTION

Commercially available Ketorolac Tromethamine eye drops, each mL of ACUVAIL® ophthalmic solution contains: ketorolac tromethamine 0.5%. Acetonitrile, Methanol, Glacial acetic acid and water used were of HPLC grade (Qualigens) were procured from local market. Ketorolac Tromethamine Working standard was procured from the Centaur Laboratories Pvt. Ltd as a gift material.

Instruments:
HPLC, Waters Alliance system with equipped Diode Array Detector and automatic injector with injection volume 50 µl. The HPLC data was analyzed with Empower-2 Software, SARTOURIUS Analytical balance with model of MSA225P-100-DA and FISHER SCIENTIFIC pH METER with model of XL15.

HPLC Conditions:
The contents of the mobile phase were mixture of Water, methanol and glacial acetic acid in the ratio 44:55:1 (v/v/v) respectively and degassed for about 10 minutes. The run time was set about 10 minutes and the column temperature was 400C. prior to injection of the Blank solution and drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The eluents were monitored at 254 nm.

Preparation of Diluent:
Water and methanol were mixed in the ratio 70:30 (v/v) respectively and degassed for about 10 minutes

Preparation of Standard stock solution: (40 mcg/ml)
A standard stock solution of the drug was prepared by 40 mg of Ketorolac Tromethamine working standard was weighed and transferred into a 50 mL volumetric flask to which 35 mL of diluent was added and sonicated to dissolve the material completely, diluted to volume with the diluent and mixed well. 5.0 mL of the above standard stock solution was pipetted into 100 mL volumetric flask, diluted to volume with diluent and mixed well.

Preparation of Standard solution: (2 mcg/ml)
5.0 mL of the above standard stock solution was pipetted into 100 mL volumetric flask, diluted to volume with diluent and mixed well.

Preparation of Sample solution: (2 mcg/ml)
4.0 mL of the sample solution (0.5% of ACUVAIL® ophthalmic Ketorolac Tromethamine solution) was taken and diluted with diluent up to 100 mL and mixed well.

Linearity:
Linearity of Detector response was established by plotting a graph of concentration (in µg/mL) versus peak area and determining correlation coefficient, y-intercept, slope, %y-intercept and residual sum of squares. Solution of Ketorolac Tromethamine was prepared in the concentration range from about Limit of Quantitation to about 150 % level of the target concentration (3 µg/mL) of Ketorolac Tromethamine and injected into the HPLC system. The detector response was found to be linear and the results were found to be within limit.

The % Recovery was calculated from the concentration data obtained from the Linearity of Detector Response against the theoretical concentration from Linearity of Detector Response was found to be within the limits. The data are presented in table 1, 2 &3.

Precision studies:
The precision of test method was evaluated by preparing six samples and analyzed as per the test procedure. 50 µL of sample solution was injected into the injector of liquid chromatograph. The retention time was found to be 3 minutes. The amount of drug present per each sample preparation was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in table 4. Typical chromatogram of Blank solution and Ketorolac Tromethamine sample solution as shown in fig 1 & 2.

Recovery Studies:
Accuracy was determined by recovery studies of Ketorolac Tromethamine, known amount of standard was added to the preanalysed sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in table 5. The study was done from about Limit of Quantitation to about 150 % level of the test concentration at three different sample preparations for each level.

Table I: Linearity data

% of Linearity Level	Concentration (µg/mL)	Peak Area
LOQ	0.0150	677
20	0.4000	17934
50	1.0000	44556
80	1.5999	71386
100	1.9999	89652
120	2.3999	106966
150	2.9999	133457
Correlation coefficient, NLT 0.997	0.999	
Y-intercept	111.314	
% Y-intercept	0.12	
Slope	44538.321	
Residual sum of square	310322.267	

Table 2: Linearity of Recovery data

% Linearity Level	Theoretical concentration (µg/mL)	Recovered concentration (µg/mL)	% Recovery
LOQ	0.0150	0.0152	101
20	0.4000	0.4033	101
50	1.0000	1.0019	100
80	1.5999	1.6052	100
100	1.9999	2.0159	101
120	2.3999	2.4053	100
150	2.9999	3.0009	100

Table 3: Linear Regression of Calibration curve

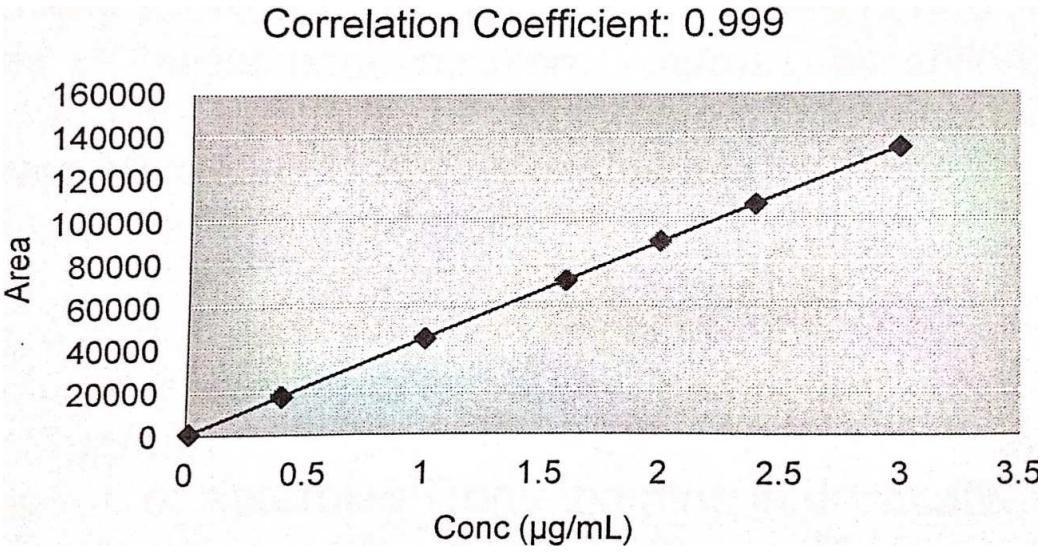


Table 4: Results of Precision studies

Sample Preparations	% Assay of Ketorolac Tromethamine	
	Analyst-1	Analyst-2
Preparation – 1	100.5	100.2
Preparation – 2	100.6	99.1
Preparation – 3	100.4	99.9
Preparation – 4	99.8	99.1
Preparation – 5	99.3	99.6
Preparation – 6	100.4	100.0
Individual Average	100.2	99.7
Individual (% RSD), NMT 2	0.5	0.5
Overall Average	99.9	
Overall % RSD, NMT 2	0.5	

Table 5: Results of Recovery studies:

Recovery Level	Preparations	% RECOVERY			
		Injection-1	Injection-2	Mean	Average
LOQ Level	Preparation -1	99.1	99.2	99.2	99.3
	Preparation -2	99.0	99.1	99.1	
	Preparation -3	99.5	99.5	99.5	
50% Level	Preparation -1	99.4	99.4	99.4	99.3
	Preparation -2	99.0	99.2	99.1	
	Preparation -3	99.2	99.3	99.3	
100% Level	Preparation -1	99.5	99.6	99.6	99.4
	Preparation -2	99.1	99.2	99.2	
	Preparation -3	99.4	99.2	99.3	
150% Level	Preparation -1	99.7	99.5	99.6	99.4
	Preparation -2	99.1	99.2	99.2	
	Preparation -3	99.5	99.5	99.5	

Table 6: Validation Summary

System suitability parameters	Observed Values	
	Analyst-1	Analyst-2
USP Tailing factor for Ketorolac peak in standard solution	1.1	1.0
Relative Standard Deviation for peak areas of Ketorolac from six replicate injections of standard solution	0.1	0.1
USP Plate count for Ketorolac peak in standard solution	6958	4891

Fig 1: Typical Chromatogram of Blank solution by HPLC

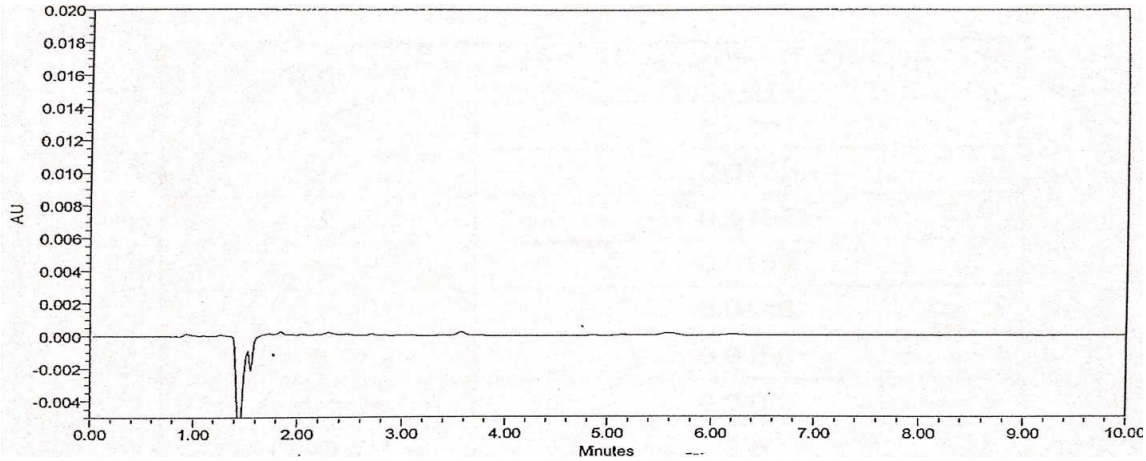
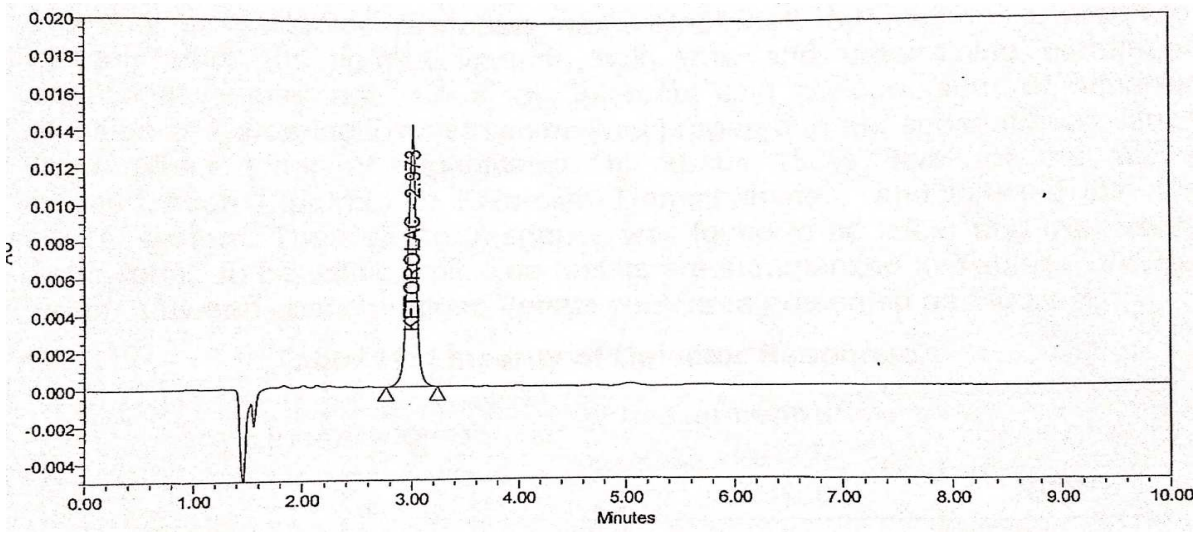


Fig 2: Typical Chromatogram of Ketorolac sample solution by HPLC



RESULTS AND DISCUSSION

The system suitability tests were carried out on freshly prepared standard solution of Ketorolac Tromethamine. The parameters studied to evaluate the suitability of the system are given in table 6.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) for Ketorolac Tromethamine were found to be 0.0049 mcg/mL and 0.0150 mcg/mL respectively. The signal to noise ratio is 3 for LOD and 10 for LOQ.

The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Ketorolac drug in Ketorolac Tromethamine eye drops. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (r2=0.999) was observed between the concentration range of 0.015 mcg/mL to 3 mcg/mL. Low values of standard deviation are indicative of the high precision of the method. The assay of Ketorolac Tromethamine eye drops was found to be 99.9 %. From the recovery studies it was found that the Ketorolac Tromethamine was recovered which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in eye drops.

CONCLUSION

The developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control testing of this dosage form of Ketorolac Tromethamine within a short analysis time.

Acknowledgements

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