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Development and validation of RP-HPLC method for determination of ketorolac tromethamine from bulk and pharmaceutical formulations

Sarita Pawar
Department of Pharmaceutical Chemistry, Sanjivani College of Pharmaceutical Education and Research, Kopargaon-423601, Maharashtra, India
Corresponding author email: gordesarita@gmail.com

Aditi Punekar
Department of Pharmaceutical Chemistry, Sanjivani College of Pharmaceutical Education and Research, Kopargaon-423601, Maharashtra, India

Seema Gosavi
Department of Pharmaceutical Chemistry, Sanjivani College of Pharmaceutical Education and Research, Kopargaon-423601, Maharashtra, India

Prasad Gorde
Department of Pharmaceutical Chemistry, Sanjivani College of Pharmaceutical Education and Research, Kopargaon-423601, Maharashtra, India

Shilpa Harak
Department of Pharmaceutical Chemistry, Gokhale Education Society' Sir Dr M S Gosavi College of Pharmaceutical Education and Research, College Road, Nashik Maharashtra, India

***Abstract*---**A simple, rapid, specific, precise and accurate Reverse phase high performance liquid chromatographic (RP-HPLC) method was developed for quantitative analysis of Ketorolac tromethamine in bulk and marketed formulations such as tablet, ophthalmic solution and gel and validated as per ICH guidelines. Chromatographic separation was performed on a C18 column using an optimized mobile phase of Methanol: Water (75:25) at a flow rate of 1ml/min using the HPLC method. The detection was done at 319 nm and Ketorolac tromethamine had a retention time 2.522 minutes. The total run time of analysis was 10minutes. The drug obeyed Beers law in the concentration range of 4-12µg/ml and the calibration plot revealed a linear relationship. The LOD and LOQ for ketorolac tromethamine

were determined to be 0.415 µg/ml and 1.3 µg/ml, respectively. The accuracy and repeatability studies of the proposed method was found to be in acceptable limits, %RSD was less than 2. The proposed method showed excellent linearity, specificity, accuracy, precision, LOD, LOQ and system suitability results within the acceptance criteria. This method with features of less run time and lower retention time can be used for routine analysis of Ketorolac tromethamine in bulk and pharmaceutical dosage forms and does not require any complicated methods.

***Keywords*---**ketorolac tromethamine, RP-HPLC, method development

Introduction

Non-steroidal anti-inflammatory medications (NSAIDs) are prescribed to treat pain and inflammation caused by musculoskeletal and joint problems, as well as surgical operations. Because of the high prevalence of rheumatoid illnesses in India, these medications are widely used. Many NSAIDs have been developed, one of which being Ketorolac Tromethamine (Figure 1) [1]. Ketorolac tromethamine is chemically (±)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid, 2- amino-2-(hydroxymethyl)-1,3-propanediol. It is also a potent antipyretic and anti-inflammatory [2]. By preventing arachidonate binding, ketorolac (KTR), like other NSAIDs, inhibits both cyclooxygenase (COX) isoenzymes, COX-1 and COX-2. The suppression of prostaglandin synthesis has been linked to anti-inflammatory and analgesic effects [3,4].

According to a literature review, there are different UV, RP-HPLC, and HPTLC methods for determining ketorolac tromethamine in conjunction with other medications such as Ofloxacin, Toradol, and others [5-9]. However, there were few easy and quick ways for determining ketorolac tromethamine individually using the RP-HPLC method [10-12]. To the best of our knowledge no single RP-HPLC method is available for the determination of the ketorolac tromethamine from the different formulation such as Tablet, Ophthalmic solution and Gel. Hence to check the applicability of the developed method we used the different marketed formulations. The present work describes new, simple, rapid, accurate, sensitive, robust and reproducible method for the determination of the ketorolac tromethamine from various marketed formulations and validation as per ICH guidelines.

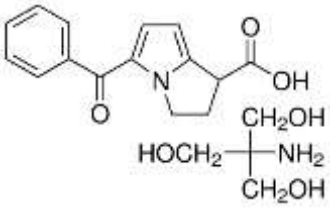

Figure 1: Structure of Ketorolac tromethamine

Materials and Methods

Instrumentation

The separation was carried out on Shimadzu (Prominence-I) HPLC system with Photodiode array detector, automatic injector, Lab solutions software and C18 (250mmx4.6mm, 5 µm) column. Weighing was done on Endel Digital analytical balance of model EJB-FE300. Shimadzu 1800 UV spectrophotometer was used for determination of detection wavelength.

Chemicals and Reagents

Analytically pure sample of Ketorolac tromethamine was obtained from Sun Pharmaceuticals, Vadodara, Gujarat. HPLC grade Methanol, Acetonitrile, Isopropyl alcohol were purchased from Merck Laboratories. Mili Q Water was used throughout the experiments. Three different pharmaceutical formulations [A (10 mg tablet), B (0.4% ophthalmic solution), C (20 mg/g Gel)] containing Ketorolac tromethamine were purchased locally.

Selection of mobile phase

Initially, to estimate Ketorolac tromethamine, number of mobile phases in different proportions were tried. The factors like HETP, system suitability, Tailing, Retention time, number of theoretical plates were considered while optimizing the mobile phase. Methanol: Water (75:25) was found to be most suitable and run as isocratic system. The mobile phase was filtered through 0.45micron filter paper followed by Sonication for degassing. Flow rate employed for analysis was 1ml/min.

Preparation of Standard Stock Solution

Accurately weighed 10 mg of Ketorolac tromethamine was transferred into 100 mL volumetric flask and dissolved in 50mL of diluents. The contents were sonicated to assure complete dissolution and final volume was made up to mark with diluents. The concentration of Ketorolac tromethamine was 100 ppm.

Preparation of different solutions

A 0.2ml, 0.4ml, 0.6ml, 0.8ml, 1.0ml and 1.2ml of standard stock solution were taken separately in 10ml volumetric flask and volume was made up to 10ml with diluent to produce final concentration of 2µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, 10 µg/ml and 12 µg/ml for drug.

Chromatographic Conditions

At 40°C, chromatographic separation was obtained, detection was performed at 319nm with a flow rate of 1 ml/min, and the run time was 10 minutes. Methanol: Water was kept as the mobile phase (75:25). The column was equilibrated for 60 minutes with mobile phase flowing through the apparatus prior to injection. Chromatographic conditions and chromatogram for the optimized method shown in Table 1 and Figure 2.

Table 1: Optimized chromatographic conditions

Column	C18 ODS (250 × 4.6 mm, 5µm)
Pump Mode	Low Pressure Gradient
Flow Rate	1ml/min
Injection volume	20 µl
Column Temperature	40^0 C
Retention Time	2.52 min
Theoretical Plates	2589

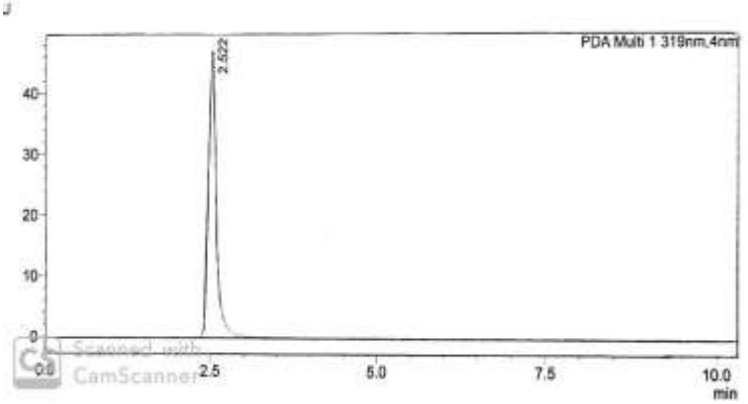

Figure 2. Chromatogram for optimized method

Linearity and calibration curve

To establish the linearity of analytical method, a series of dilution ranging from 4 to 20 µg/ml was prepared. All the solutions were filtered through 0.2 µ membrane filter and injected, chromatograms were recorded at 319nm and it was repeated thrice. A calibration curve was plotted between mean peak area and respective concentration. The regression equation was derived.

Validation of developed method

Linearity

The capacity of an analytical process to produce test results that are directly proportional to the concentration (quantity) of analyte in the sample (within a specific range) is known as linearity. Five replicate injections of the standard stock solution (4- 12 µg/ml) was made and mean area was determined. The correlation coefficient and linear regression equation for Ketorolac tromethamine were calculated. The response factor was calculated by dividing the mean area by the respective concentration. The results are shown in Table 2

Table 2: Results for linearity

Parameter	HPLC
Linearity Range	4- 12 µg/ml
Correlation Coefficient	0.996

Slope	40940
Intercept	17950

Specificity

The capacity of an analytical method to quantify an analyte accurately in the presence of interferences that may be present in the sample matrix is known as specificity. Chromatographic blanks (from a sample known to have no analyte) are examined in the expected time range of the analyte peak to ensure specificity.

Precision

The precision of an analytical method refers to the degree of agreement between a set of measurements obtained by multiple sampling of the same homogeneous sample under similar analytical conditions. The repeatability was performed for five replicates at three concentrations in linearity range (4-12 µg/ml) of Ketorolac tromethamine indicating the precision under the same operating conditions over short interval time. Results were shown in Table 3 & 4.

Table 3: Results for Inter-day and Intraday Precision

Sr.No	Concentration (µg/ml)	% RSD	
		Intra-Day	Inter-Day
1	4	1.58	1.52
2	8	1.44	1.02
3	12	1.25	1.29

Table 4: Results for Repeatability

Sr. No.	Concentration (µg/ml)	Peak Area
1	8	349082
2	8	347119
3	8	349711
4	8	351664
5	8	351100
	SD	801.707
	RSD	0.22

Accuracy

Recovery tests were performed on pre-examined samples using the standard addition method at 50, 100, and 150 percent levels, with subsequent analysis to validate accuracy of the method. At each level one determination were performed. Results of recovery study for ketorolac tromethamine in tablet, ophthalmic solution and gel are shown in Table 6.

Table 6: Results for Accuracy

Type of Formulation	Percentage (%)	Added conc. of solution	Observed conc. of solution	% Recovery

		(µg/ml)	(µg/ml)	
Tablet	50	12	12.05	100.65
	100	16	16.03	100.3
	150	20	20.23	101.91
Ophthalmic Solution	50	12	11.58	96
	100	16	15.92	99
	150	20	20.97	102
Gel	50	12	12.07	100.8
	100	16	16.2	102
	150	20	19.92	99

Robustness

The flow rate was varied by 0.2 mL/min, resulting in 0.8 mL/min and 1.2 mL/min, respectively. A 10 µg/ml standard solution of ketorolac tromethamine was injected at each flow rate. The retention time reduced as the flow rate increased, and vice versa. SD of the robustness for ketorolac was calculated. Results were shown in Table 6

Table 6: Results for Robustness

Sr. No.	Concentration (µg/ml)	Peak Area at Flow Rate 0.8 ml/min	Retention Time(min)	Peak Area at Flow Rate 1.2 ml/min	Retention Time(min)
1	10	606957	2.609	596444	2.385
2	10	618131	2.729	581278	2.340
3	10	613211	2.714	572121	2.391
SD		4021.17		7473.89	
RSD		0.67%		1.2 %	

Limit of Detection and Limit of Quantitation

The LOD and LOQ of developed method were determined based on the standard deviation of response and slope of the linearity curve. LOD and LOQ were found to be 0.41 µg/ml and 1.32 µg/ml respectively.

Results and Discussion

HPLC method was developed and validated for the estimation of Ketorolac Tromethamine in three different dosage forms. The linearity of analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Wavelength of detection 319nm was selected on the basis of results obtained in preliminary UV spectrophotometric analysis. The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (50%, 100% and 150%) was found at three replicates and three concentration levels. The value of % means just close to 100, SD and % RSD less than 2, indicate the accuracy of method.

Precision was determined by repeatability and intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analysts. The value of SD and %RSD less than 2 indicate the precision of method. The results of the analysis of three different dosage forms containing Ketorolac Tromethamine were reported. The assay value of drug was close to 100, SD and %RSD less than 2 indicate no interference of excipients in the estimation of drugs (13,14).

Conclusion

The proposed HPLC method was validated as per the International Conference on Harmonization (ICH) Q2B guidelines and was found to be applicable for routine quantitative analysis of Ketorolac Tromethamine by HPLC in pharmaceutical dosage form. The results of linearity, precision, accuracy and specificity, were proved to be within the limits. The method provides selective quantification of Ketorolac Tromethamine with no interference from other formulation excipients. The proposed method was highly reproducible, reliable, rapid, robust and specific. Therefore, a high percentage of recovery and the run time less than five minutes allow its application for the routine determination of Ketorolac Tromethamine in the pharmaceutical dosage form.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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