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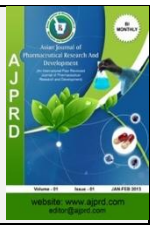

Open Access Research Article

Development and Validation of RP-HPLC Method for Oral Printed Films of Ketorolac Tromethamine

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A B S T R A C T

Abstract:

For the analysis of Ketorolac Tromethamine in Oral film formulation, a simple, precise, and accurate method was developed and validated. On a Kromosil C18 column (150 cm 4.6 mm 5), an isocratic HPLC analysis was performed. The chemical was isolated using a solution of A 55:45 V/V methanol and ammonium dihydrogen phosphate buffer with a pH of 3.0 was used. Adjusted with 1.5 mL/min flow of O-phosphoric acid as the mobile phase UV detection was carried out. Photo diode array detection was used at 314 nm. The retention time was discovered to be 6.08 minutes. The system suitability criteria, such as theoretical plate count, tailing, and percentage, should be kept to a minimum. The RSD between six standard injections was within the acceptable range. The procedure was tested and found to be valid. According to the ICH guidelines over the concentration range of 50-150 gm/L, calibrations were linear. The correlation coefficient (r) of 0.999 indicates this. The method's resilience was tested by systematically changing the chromatographic settings. The approach described may be used for routine quantitative analysis.

Keywords: Ketorolac Tromethamine, RP-HPLC Method, PDA detector.

A R T I C L E I N F O: Received 2022; Review Complete 2022; Accepted 2022; Available online 15 Oct. 2022

Cite this article as:

Dule P, Kilor V, Development and Validation of RP-HPLC Method for Oral Printed Films of Ketorolac Tromethamine, Asian Journal of Pharmaceutical Research and Development. 2022; 10(5):45-52.

DOI: http://dx.doi.org/10.22270/ajprd.v10i5.1170

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INTRODUCTION:

Orodispersible is the term referred to dosage form which disperse or disintegrate into mouth i.e. oral cavity[1]. The time required to disintegrate should be not more than 3 minutes.An ideal oral films should have sufficient flexibility, elasticity, and softness, as well as the ability to resist breaking, disintegration time, and flavor compliance[2]. During the formulation development stage and the requisite standard protocols, all of these parameters must be examined. Through define and analyse orodispersible films, a variety of approaches may be used, ranging from physical and mechanical characteristics to in vitro disintegration to in vivo drug release in people. This study discusses the numerous in vitro and in vivo methodologies used by pharmaceutical companies, regulatory agencies, and drug delivery researchers to assess the physical and mechanical characteristics of oral films. Non-steroidal anti-inflammatory medications (NSAIDs) are used to treat pain and inflammation (Sunil et al. 2017)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. Ketorolac Tromethamine is one of many NSAIDs that have been commercialized. Chemically, it is 2-amino-2-(hydroxymethyl) propane-1,3-diol;5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid (**Fig.1**). Ketorolac printed films are new formulation manufactured in RnD area of Zim Laboratories limited. Further analytical work was carried out. Ketromac DT Tablet are used as standard formulation. Simultaneous spectrophotometric measurement of Ofloxacin and Ketorolac Tromethamine in tablet dosage forms is one of several studies for the

estimation of the Ketorolac Tromethamine medicine utilising multiple methodologies[3]. Formulation and tablet for treatment with super disintegrants. Ketorolac Tromethamine was used in the formulation and development of an enteric coated dosage form. HPLC technique for simultaneous measurement of Moxifloxacin Hydrochloride and Ketorolac Tromethamine in pharmaceutical dosage form that shows stability[4]. Ketorolac Tromethamine revalidation and analytical assessment by HPTLC employing reflectance scanning densitometry. Ketorolac Tromethamine Gel with Genipin for Periodontal Diseases: Preparation and Evaluation. UV-Visible spectroscopy was used to estimate Moxifloxacin HCL and Ketorolac Tromethamine in their combination dose form using the absorption ratio approach[5]. Ketorolac determination in real time Validated Reversed Phase High Resolution Imaging of Tromethamine and Furosemide in Intestinal Perfusion Samples Liquid Chromatography with High Performance. Formulation and assessment of a Ketorolac is a Non-steroidal Anti-Inflammatory Drug (NSAID) with an ocular administration method.

Tromethamine is based on the idea of in situ pH-triggered gelation. Ketorolac's voltametric behaviour and HPLC-EC determination.

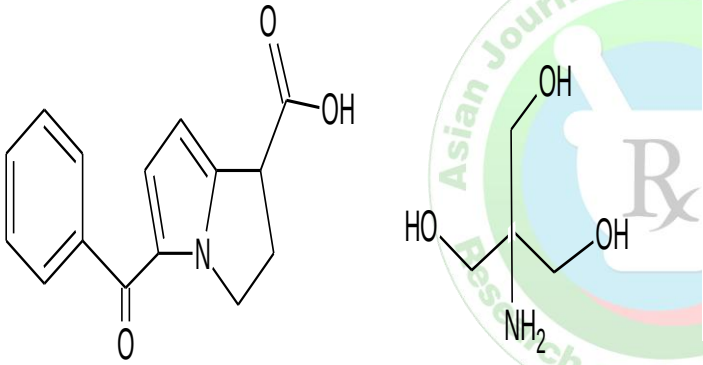

Figure 1: Chemical Structure of Ketorolac Tromethamine

EXPERIMENTAL PROCEDURES

Instrument:

We used Schimadzu system with a 22996 PDA detector, a Sartorius Electronic Analytical balance, a Crest sonicator, and a Kromosil C18 column (150 cm 4.6 mm 5).

Chemicals and Reagents

Ketorolac Tromethamine was got as a free gift sample from Zim Laboratories Limited in Nagpur. In the studies, a pharmaceutical product (Ketorolac tromethamine Oral films) with the same quantity of drug formulations was employed. Zim Laboratories provided HPLC quality methanol and ammonium dihydrogen phosphate. Throughout the experiment, HPLC grade deionized water was utilized. [6]

assessment of a Ketorolac Tromethamine mouth dissolving

Buffer preparation:

5.75 grams of ammonium dihydrogen phosphate were dissolved in 1000 milliliters of distilled water, and the pH was corrected to 3.0 using O-phosphoric acid. It was degassed and filtered using a 0.45 m nylon membrane filter. It was utilized as diluents in the sample and standard preparation[7-9].

METHOD

Wavelength detection

In a 100 ml volumetric flask, 100 mg of Ketorolac Tromethamine was accurately weighed, 100 ml of methanol was added, sonicated for 5 minutes, and filtered through a 0.45 m nylon membrane filter. Pipette out 1 mL of the aforementioned solution, dilute to 10 mL with methanol in a 10 mL volumetric flask, and scan with UV spectroscopy between 200 and 400 nm (**Fig. 2**)[10-12].

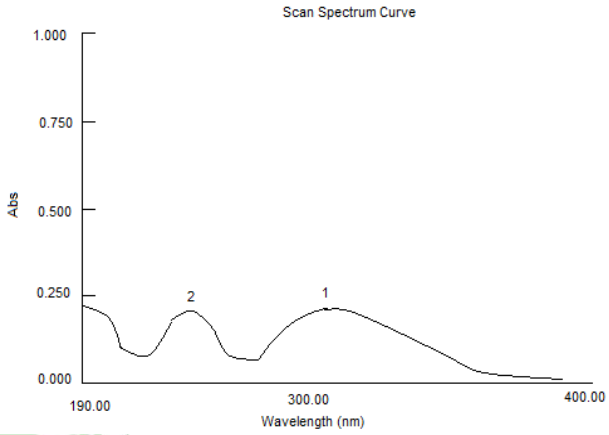

Figure 2: UV Absorption Spectra

Chromatographic condition

At 30° C, chromatographic separation was obtained, detection was performed at 314 nm with a flow rate of 1.5 mLmin-1, and the run time was held at 20 minutes. The column was equilibrated for 60 minutes with the mobile phase running through the apparatus prior to the injection of drug solution. The injection volume for the test level was 10 litres. To test for solvent interference, a blank containing the mobile was injected [13].

Standard preparation

Weighing 100 mg of Ketorolac Tromethamine into a 100 mL volumetric flask and adding diluent to make up the capacity. 1 mL of the aforesaid solution is pipette out and 10 mL of mobile phase is added to produce a final concentration of 100 gm/L. **Figure 3** shows a typical chromatogram of the standard.

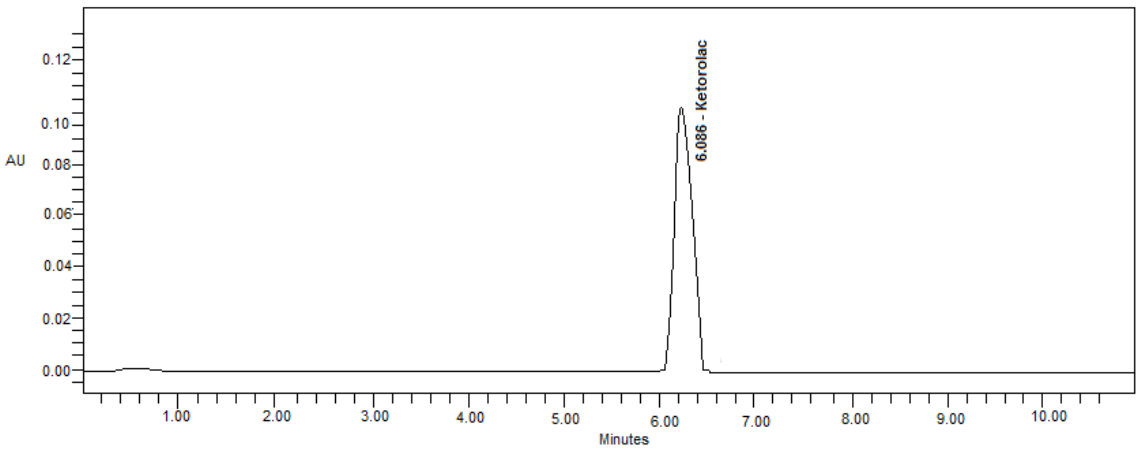

Figure 3: Standard chromatogram of Ketorolac

Sample preparation

The equivalent of 20 mg of Ketorolac Tromethamine in ACULAR LS & ACULAR PS ophthalmic sample solution was weighed and transferred to a 100 mL volumetric flask. 70 mL mobile phase was added to the aforementioned solution, which was sonicated for roughly 15 minutes before being filtered through a 0.45 m nylon membrane filter. With mobile phase, 1.25 mL of the filtrate was diluted to 10 mL. **Figure 4** and **Table 1** show a typical chromatogram of the sample.

Table 1: Analysis of Marketed Formulation

Formulation	Ingredients	Ingredients Labeled amount (mg)	Amount found (mg) n=6	Found %
Ketorolac Printed films	Ketorolac Tromethamine	10	0.429	102%
Ketromac DT Tablet	Ketorolac Tromethamine	10	0.418	101%

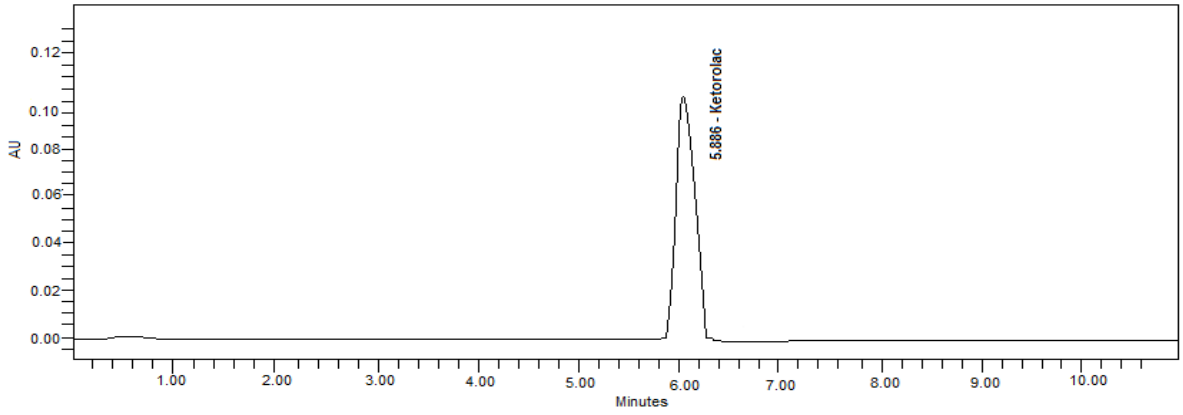

Figure 4: Test chromatogram of Ketorolac

Evaluation of System Suitability

The chromatograms were obtained after 10 L of standard solution was injected in six duplicates before and after the analysis. Column efficiency, plate count, and tailing factor were all recorded as system suitability metrics. The column efficiency was proven to be greater than 2000 USP plate counts, USP Tailing for the same peak was less than 2.0, and the percent RSD of six injections of the standard solution was less than 2.0 percent, as shown in **Fig. 5** and **Table 2.**

Table 2: System suitability study

Injection	RT	Peak Area	USP plate count	USP Tailing factor
1	6.0848	2612836	2121	1.276
2	6.0487	2618061	2001	1.268
3	6.0341	2517365	2148	1.218
4	6.0209	2519486	2227	1.209
5	5.9827	2517701	2158	1.207
Mean	6.03424	2557089.8	2131	1.236
SD	0.037401	61905.95	---	---
%RSD	0.006	0.0238	---	---

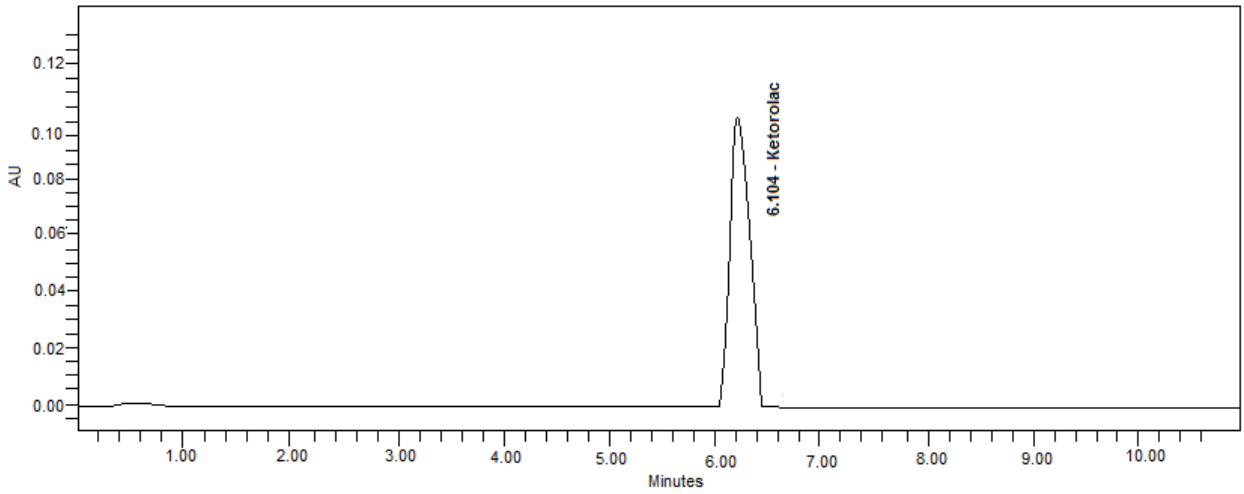

Fig 5: System Suitability Chromatogram

ANALYTICAL METHOD VALIDATION (ICH, 2005)[14-15]

Specificity

The placebo solution was made in the same way as the test solution, but with an equal weight of placebo in each part. Following the test conditions, a placebo solution was injected into the HPLC system, the chromatogram was recorded, and the responses of the peaks were measured for any excipient interference at the retention period of Ketorolac Tromethamine.

Precision

Precision was examined in terms of application and measurement repeatability.Six repetitions of the sample injection (100 gm/L) were used to test the repeatability of the standard application (system precision). Six distinct sample preparations from the same homogeneous mix of the marketed sample (100 gm/L) were tested for repeatability of sample measurement (method precision). The percentage RSD for standard preparation repeatability was 1.16 percent, whereas the percentage RSD for sample preparation repeatability was 0.02 percent. The chromatogram presented in **Fig. 6** and **Table 3** reveals that the method's accuracy is excellent, since the percentage RSD is less than 2%.

Table 3: Precision study of the method

S.No	Sample area	% Area	Amount Present (mg)
1	2647836	100.39	0.468
2	2597061	98.87	0.479
3	2528365	97.87	0.425
4	2601486	99.72	0.456
5	2579701	99.69	0.454
Mean	2590890	99.308	0.4564
SD	43092.26	0.96	0.020

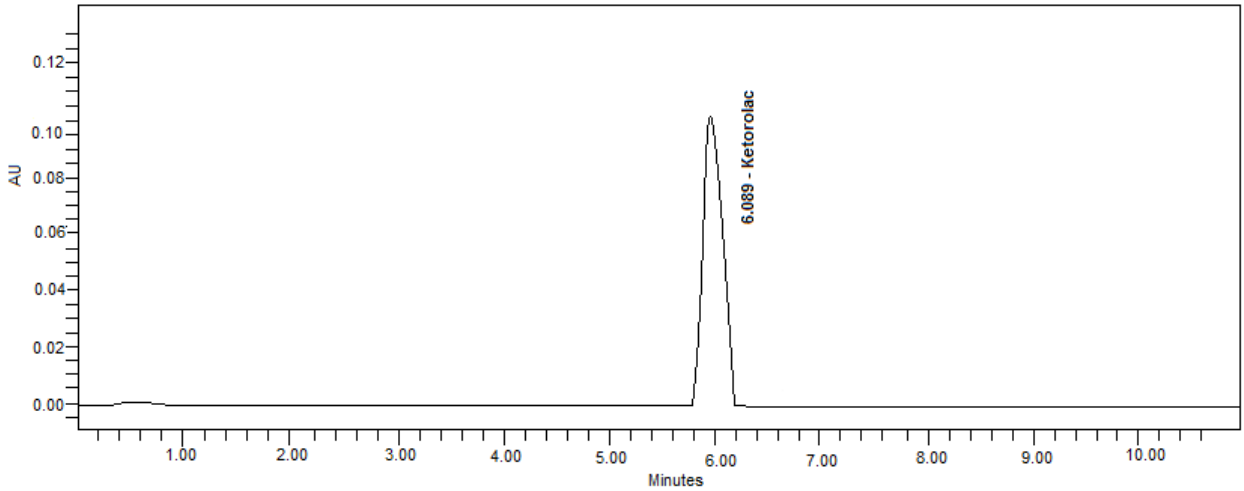

Fig 6: Precision Chromatogram

Linearity

By producing and injecting a solution with a concentration of 50-150 gm/L, the linearity of Ketorolac Tromethamine was tested. The calibration curve for Ketorolac Tromethamine shows that the response is linear across the concentration range examined, with a correlation coefficient (r) of 0.999.

Stability of sample solution

The sample solution was produced according to the test procedure and examined at various time intervals while kept at room temperature. The % response between the initial and various time periods indicates that the sample solutions were stable at room temperature for at least 24 hours (**Table 4**).

Table 4: Stability study

S. No	Time Interval	Peak response
1	0 h	2618836
2	2 h	2698571
3	6 h	2627365
4	10 h	2623486
5	16 h	2644701
6	24 h	2675739
Mean		2596031
SD		32295.38
%RSD		1.52

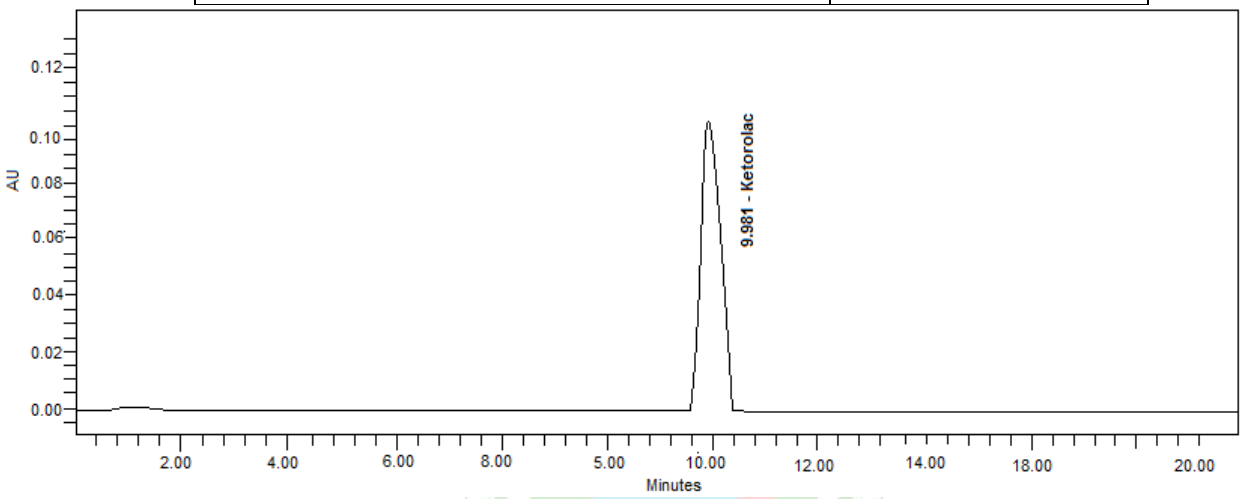

Fig 7: Robustness Chromatogram

Robustness

The method's robustness was tested by modifying certain operational analytical variables such as flow rate, column, oven temperature, detection wavelength, and mobile phase while analyzing a reference solution under normal operating settings. **Table 5** shows the situations with variation and their outcomes. The tailing factor is near unity, indicating peak symmetry, and theoretical plate counts were above 2000. Hence **Figure 7** depicts the robustness of the breadth of variations applied to analytical circumstances.

Table 5: Robustness studies

System suitability parameters (Variations)	% RSD of peak area response (n=3)	Mean tailing factor (n=3)	Mean retention time in min. (n=3)
Change in %+5	1.204	0.667	6.004
Organic Phase-5	1.224	0.859	6.049
Change in pH+3	0.0187	1.277	6.057
-3	0.0167	1.302	6.078
Change in flow +1.5	0.0034	1.219	6.755
-1.5	0.0029	1.246	6.845
Change in temp.+25	0.0027	1.188	5.177

Accuracy

In the percentage recovery tests, a known amount of pure standard medication was added to the pre-analyzed sample. A 100 mL volumetric flask was filled with a solution equivalent to 100 mg of Ketorolac Tromethamine. After that, the sample was spiked with standard at 50, 100, and 150 percent of the test concentration. The spiked sample solutions were then tested in triplicate, with the findings compared and represented as a percentage. **Table 6** and **Fig. 8** reveal that the mean % recovery of Ketorolac Tromethamine was determined to be between 98.54 and 102.5, which is within the acceptable limits.

Table 6: Accuracy Study

Spike level	Amount Added µgmL-1	Peak Area	% Recovery	% Mean Recovery
50%	0.12	1348974	101.90	100.55
50%		1347894	99.83	
50%		1329727	99.93	
100%	0.25	2578598	101.34	99.38
100%		2596802	99.12	
100%		2569358	97.68	
150%	0.36	3498611	96.94	98.94
150%		3697796	99.28	
150%		3897218	100.61	

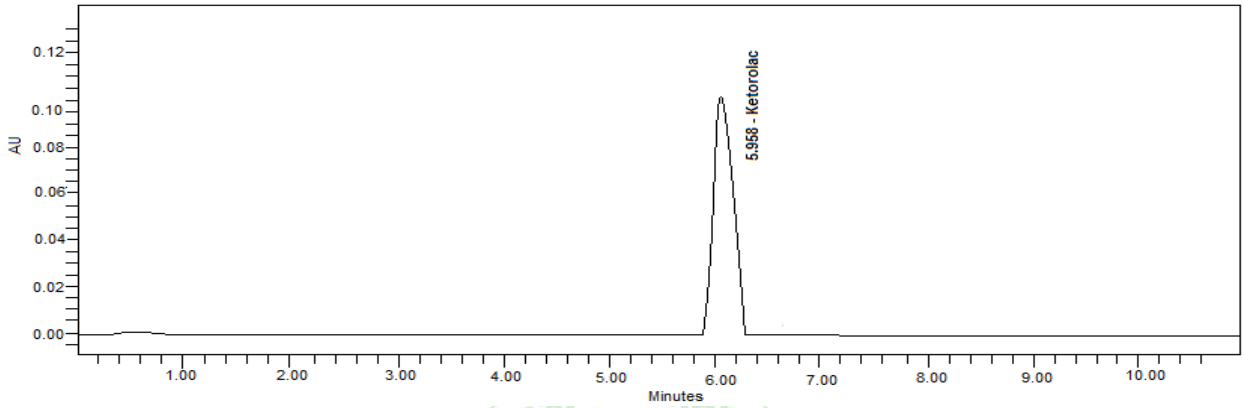

Fig 8: Accuracy Chromatogram

Limit of detection (LOD) and Limit of quantification (LOQ)

The lowest quantity of analyte in a sample that can be detected, but not necessarily quantified, under the indicated experimental circumstances is known as the limit of detection (LOD). The formulas LOD=3.3 (SD/S) and LOQ=10 (SD/S) were used to determine LOD and LOQ using the standard deviation and slope values received from the calibration curve. In **Fig. 9 & 10** and **Table 7**, the LOD and LOQ values for Ketorolac Tromethamine were determined to be 0.116 gmL^{-1} and 0.32 gmL^{-1}, respectively.

Table 7: LOD and LOQ

S. No	Concentration (µgmL-1)	Peak Area
1	50	1357956
2	70	1798523
3	100	2493790
4	120	3087955
5	150	3877114

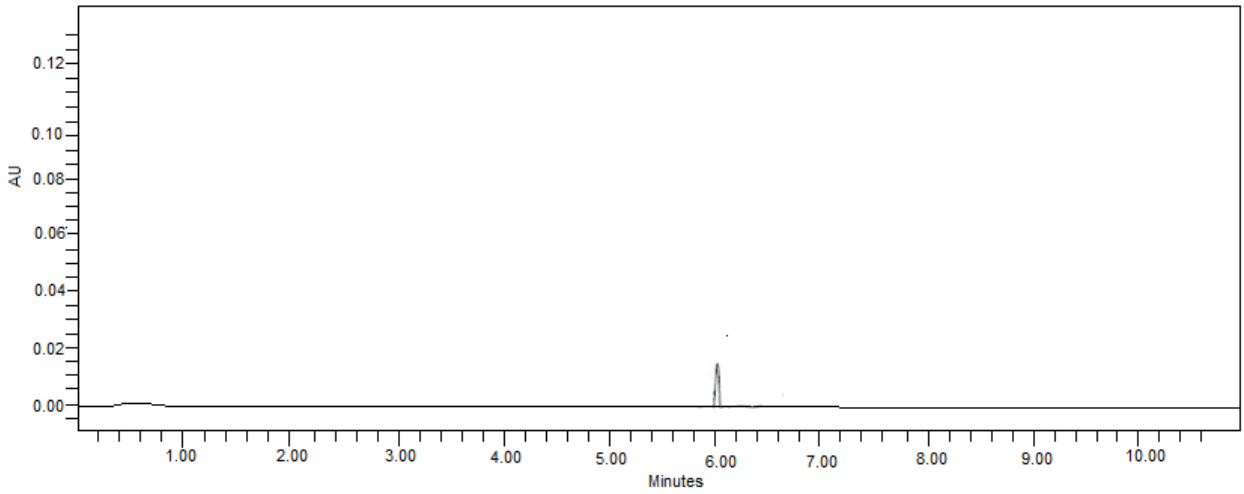

Fig 9: LOD

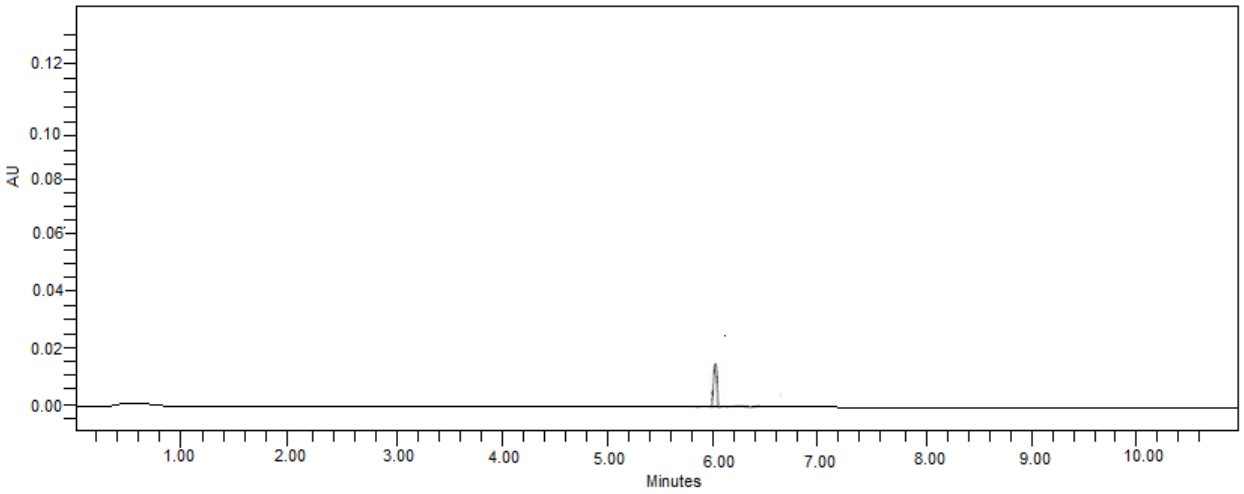

Fig 10: LOQ

RESULTS AND DISCUSSION

A mobile phase comprising ammonium dihydrogen phosphate and methanol (44:55 V/V), Kromosil C18 (150 cm x 4.6 mm x 5) column, 1.5 mLmin-1 flow rate, 10 l injection volume, 300C column oven temperature, 314 nm wavelength, and 20 min run time was determined to be appropriate for all combinations. The retention duration was 6.01 minutes under these chromatographic conditions. When the method's specificity was tested by injecting a placebo solution, no peaks were identified at the Ketorolac Tromethamine retention time. By creating a sample solution according to the suggested technique and analyzing it initially and at 1 h intervals up to 24 h while retaining the sample solution at room temperature, the stability of the sample solution was determined.The medication solution was confirmed to be stable for 24 hours at room temperature, according to the findings of the stability tests. The percent RSD for system precision and method precision was 1.16 and 0.02, respectively.Between the concentrations of 50 gm/L and 150 gm/L of Ketorolac Tromethamine, an excellent linearity connection was discovered, as shown by a correlation coefficient (r) value of 0.999. Intermediate Precision was achieved by switching the analyst, column, and chromatographic settings while keeping the findings within the limitations. The Robustness technique was tested by adjusting the method's chromatographic parameters, such as the mobile phase Methanol concentration, flow rate, column temperature, and wavelength. The tailing factor and retention time, for example, stayed within the limitations. The method's accuracy was determined, and the % recovery was computed. The standard sample was recovered at a rate of 102.5 percent on average, according to the statistics.

CONCLUSION

The Ketorolac Tromethamine technique was discovered to be a straightforward approach that does not require any experimental conditions. The approach was found to be specific, accurate, linear, exact, rugged, and robust based on the validation findings. The duration was just 20 minutes, allowing for quick quantification of a large number of samples in routine and quality control ophthalmic formulation analysis.

ACKNOWLEDGEMENT

The authors are grateful to the management and staff of Zim Laboratories, Nagpur and Gurunanak College of Pharmacy, Nagpur. We are also thankful for the DST SERB for providing fund to carry out research work.

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