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Development and validation of Ketorolac Tromethamine in eye drop formulation by RP-HPLC method

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Abstract   A simple, precise and accurate method was developed and validated for analysis of Ketorolac Tromethamine in eye drop formulation. An isocratic HPLC analysis was performed on Kromosil C_{18} column (150 cm × 4.6 mm × 5 μm). The compound was separated with the mixture of methanol and ammonium dihydrogen phosphate buffer in the ratio of 55:45 V/V, pH 3.0 was adjusted with O-phosphoric acid as the mobile phase at flow of 1.5 mL min^{-1}. UV detection was performed at 314 nm using photo diode array detection. The retention time was found to be 6.01 min. The system suitability parameters such as theoretical plate count, tailing and percentage RSD between six standard injections were within the limit. The method was validated according to ICH guidelines. Calibrations were linear over the concentration range of 50–150 μg mL^{-1} as indicated by correlation coefficient (*r*) of 0.999. The robustness of the method was evaluated by deliberately altering the chromatographic conditions. The developed method can be applicable for routine quantitative analysis.

1. Introduction

Non-steroidal anti-Inflammatory drugs (NSAIDs) are used for the management of pain and inflammation associated with musculo-skeletal, joint disorders and operative procedures.

The uses of these drugs are prevalent in India due to the high rates of occurrence of rheumatoid disorders. Many NSAIDs have been marketed, one among them is Ketorolac Tromethamine. It is chemically, (±)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid, 2-amino-2-(hydroxymethyl)-1,3-propanediol (Fig. 1). In India it is marketed under the trade name of Acular LS and Acular PS. When administered as eye drops it demonstrated analgesic, anti-histaminic, anti-inflammatory and anti-pyretic activity. The mechanism of action is to inhibit prostaglandin biosynthesis and given systemically does not cause pupil constriction.

Several studies for the estimation of the Ketorolac Tromethamine drug using various techniques have been carried out, some of them being; Simultaneous spectrophotometric
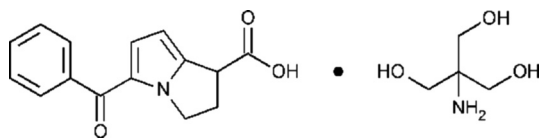
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Figure 1   Chemical structure of Ketorolac Tromethamine.

estimation of Ofloxacin and Ketorolac Tromethamine in tablet dosage forms (Fegade et al., 2009). Formulation and evaluation of mouth dissolving tablet of Ketorolac Tromethamine for the management using super disintegrants (Patil et al., 2009). Formulation and development of enteric coated dosage form using Ketorolac Tromethamine (Rupesh et al., 2010). Stability indicating the HPLC method for the simultaneous determination of Moxifloxacin Hydrochloride and Ketorolac Tromethamine in pharmaceutical dosage form (Naeem et al., 2011). Revalidation and analytical evaluation of Ketorolac Tromethamine by HPTLC using reflectance scanning densitometry (Rao et al., 2011). Preparation and evaluation of Ketorolac Tromethamine Gel containing genipin for periodontal diseases (Jeon et al., 2007). Absorption ratio method for the estimation of Moxifloxacin HCl and Ketorolac Tromethamine in their combined dosage form by UV–Visible spectroscopy (Gandhi et al., 2011). Simultaneous determination of Ketorolac Tromethamine and Furosemide in intestinal perfusion samples by validated Reversed Phase High Performance Liquid Chromatography (Gupta et al., 2005). Formulation and evaluation of an ophthalmic delivery system of the Non-steroidal Anti-Inflammatory Drug (NSAID), Ketorolac Tromethamine based on the concept of pH-triggered in situ gelation (Kugalur et al., 2010). Voltametric behavior of Ketorolac and its determination in HPLC-EC (Squella et al., 2006).

2. Experimental procedures

2.1. Instruments

Waters 2695 with 22996 PDA detector HPLC systems (Empower 2 software), Sartorius Electronic Analytical balance, Crest sonicator and Kromosil C$_{18}$ column (150 cm × 4.6 mm × 5 μ) were used.

2.2. Chemicals and reagents

Free gift sample of Ketorolac Tromethamine was obtained from Spectra Pharma Research Laboratories, Hyderabad. A pharmaceutical product (ACULAR LS and ACULAR PS eye drops) composition containing the same amount of drug formulations was used in the experiments. HPLC grade methanol and ammonium dihydrogen phosphate were procured from Merck Laboratories, Mumbai. HPLC grade deionized water was used throughout the experiment.

2.3. Buffer preparation

Ammonium dihydrogen phosphate (5.75 g) was dissolved in 1000 mL of distilled water and adjusted the pH to 3.0 with O-phosphoric acid. It was filtered through 0.45 μm nylon membrane filter and degassed. It was used as diluents for the preparation of sample and standard.

3. Method

3.1. Wavelength detection

Accurately weighed Ketorolac Tromethamine equivalent to 100 mg in 100 ml volumetric flask, 100 ml of methanol were added, sonicated for 5 min and filtered through 0.45 μm nylon membrane filter. Pipette out 1 mL of the above solution and dilute to 10 mL with methanol in 10 mL volumetric flask and scanned between 200 and 400 nm by UV spectroscopy (Fig. 2).

3.2. Chromatographic conditions

Chromatographic separation was achieved at 30 °C, the detection was carried at 314 nm at a flow rate of 1.5 mL min^{-1} and run time was kept at 20 min. Prior to the injection of drug solution column was equilibrated for 60 min with the mobile phase flowing through the system. The injection volume was 10 μL for assay level. Blank containing the mobile was injected to check the solvent interference.

3.3. Standard preparation

Ketorolac Tromethamine (100 mg) was weighed and transferred into a 100 mL volumetric flask and make up to the volume with diluent. Pipette out 1 mL of the above solution is diluted 10 mL with the mobile phase to get the final concentration of 100 μg mL^{-1}. A representative chromatogram of the standard was shown in Fig. 3.

3.4. Sample preparation

ACULAR LS and ACULAR PS ophthalmic sample solution equivalent to 20 mg of Ketorolac Tromethamine was weighed and transferred to 100 mL volumetric flask. To the above solution was added 70 mL of the mobile phase, sonicated for about 15 min and filtered through 0.45 μm nylon membrane filter. 1.25 mL of the filtrate was diluted to 10 mL with the mobile phase. A representative chromatogram of the sample was shown in Fig. 4 and Table 1.
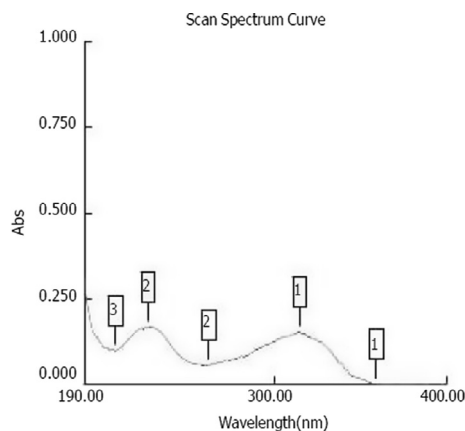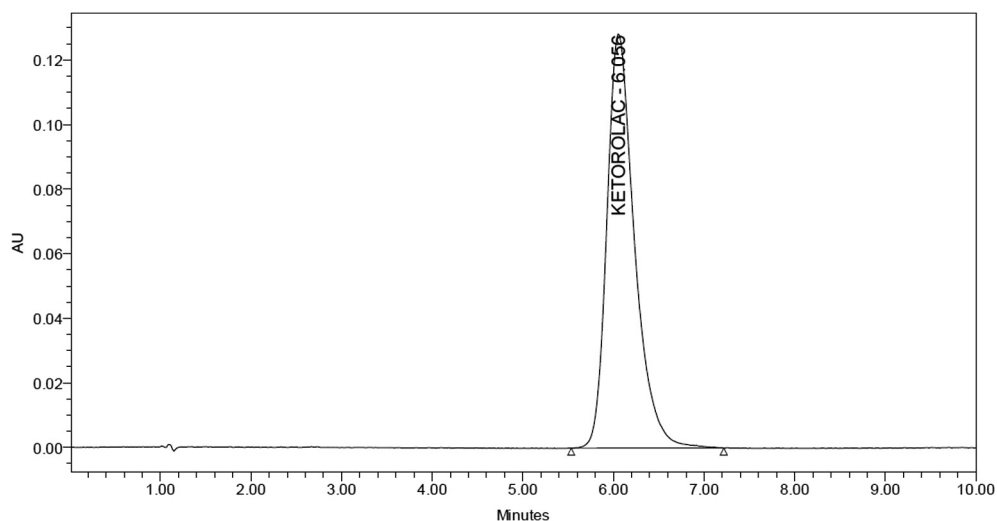

Figure 2   UV absorbance spectra.
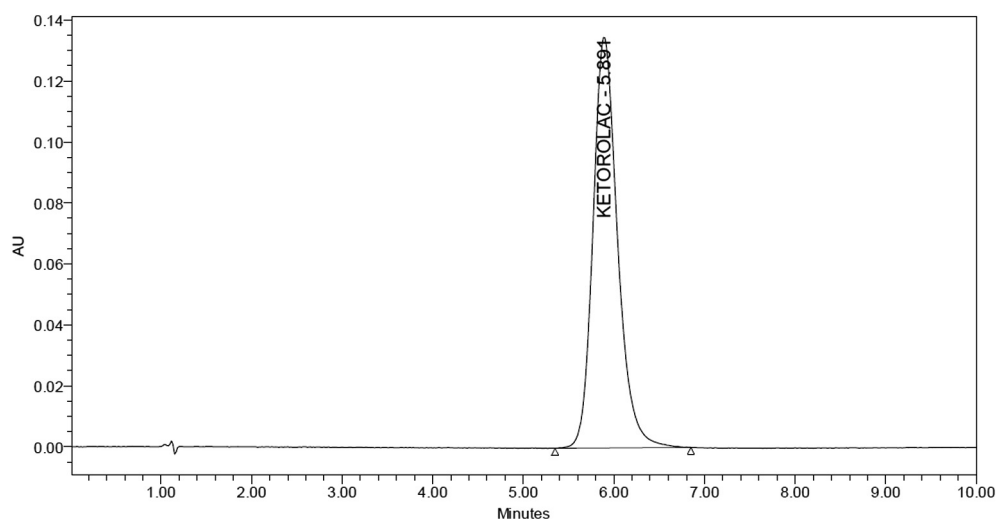
Figure 3 Standard chromatogram.

Figure 4 Test chromatogram.

Table 1 Analysis of marketed formulations.

Commercial formulation	Ingredients	Labeled amount (mg)	Amount found (mg) $n = 6$	Found in (%)
Acular LS eye drops	Ketorolac Tromethamine	0.4	0.43	102
Acular PF eye drops	Ketorolac Tromethamine	0.4	0.42	101

3.5. Evaluation of system suitability

10 μL of standard solution was injected in six duplicate before and after the analysis and the chromatograms were recorded. System suitability parameters like column efficiency, plate count and tailing factor were also recorded. The column efficiency determined was found to be more than 2000 USP plate count, USP Tailing for the same peak is not more than 2.0 and % RSD of six injection of the standard solution is not more than 2.0% the chromatogram as shown in Fig. 5 and Table 2.

4. Analytical method validation (ICH, 2005)

4.1. Specificity

Placebo solution was prepared as per the test solution using equivalent weight of the placebo in a portion. Placebo solution was injected into the HPLC system following the test conditions, the chromatogram was recorded and measured the responses of the peaks were noted for any interference of the excipient at the retention time of Ketorolac Tromethamine (Fig. 6).
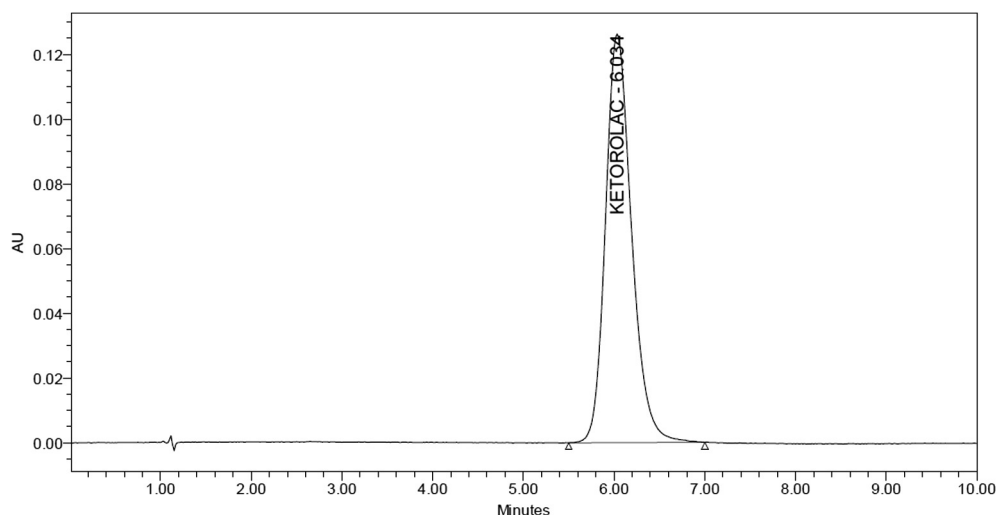
Figure 5 System suitability chromatogram.

Table 2 System suitability study.

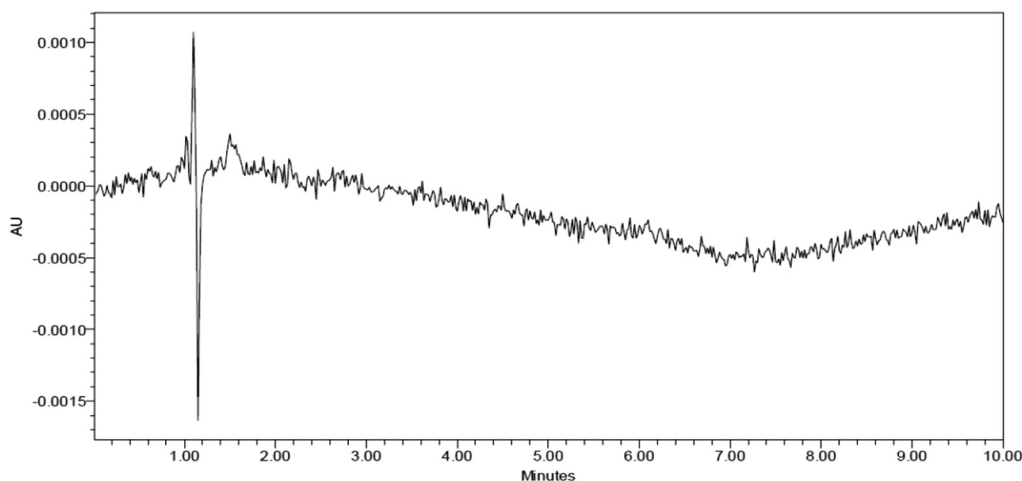| Injection	RT	Peak area	USP plate count	USP tailing factor
1	6.085	2606836	2011	1.28
2	6.049	2698061	2008	1.27
3	6.034	2530365	2176	1.22
4	6.021	2591486	2218	1.21
5	5.983	2572701	2136	1.25
Mean	6.0344	2596031.33	2108	1.24
SD	0.037401	61905.95	–	–
% RSD	0.006	0.0238	–	–

Figure 6 Placebo (Specificity) chromatogram.

4.2. Precision

Precision was measured in terms of repeatability of application and measurement. Repeatability of standard application (system precision) was carried out using six replicates of the sample injection (100 µg mL^{-1}). Repeatability of sample measurement (method precision) was carried out in six different sample preparations from the same homogenous blend of the marketed sample (100 µg mL^{-1}). The percentage RSD for repeatability of standard preparation was 1.16% whereas the % RSD for repeatability of the sample preparation was 0.02%. This shows that the precision of the method is satisfactory as percentage RSD is not more than 2% the chromatogram as shown in Figs. 7 and 8 and Table 3.

4.3. Linearity

The linearity of Ketorolac Tromethamine was determined by preparing and injecting a solution with a concentration of about 50–150 µg mL^{-1}. The calibration curve indicates the response is

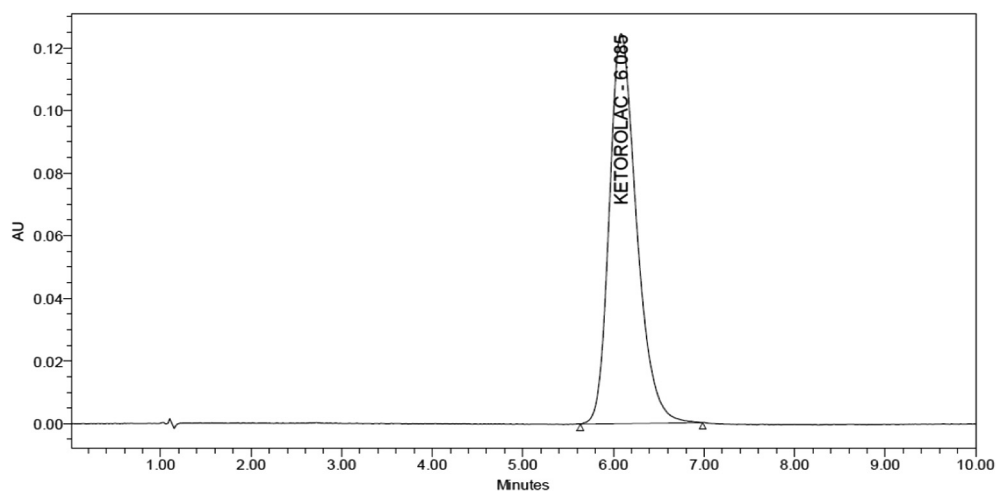**Figure 7** Precision chromatogram.

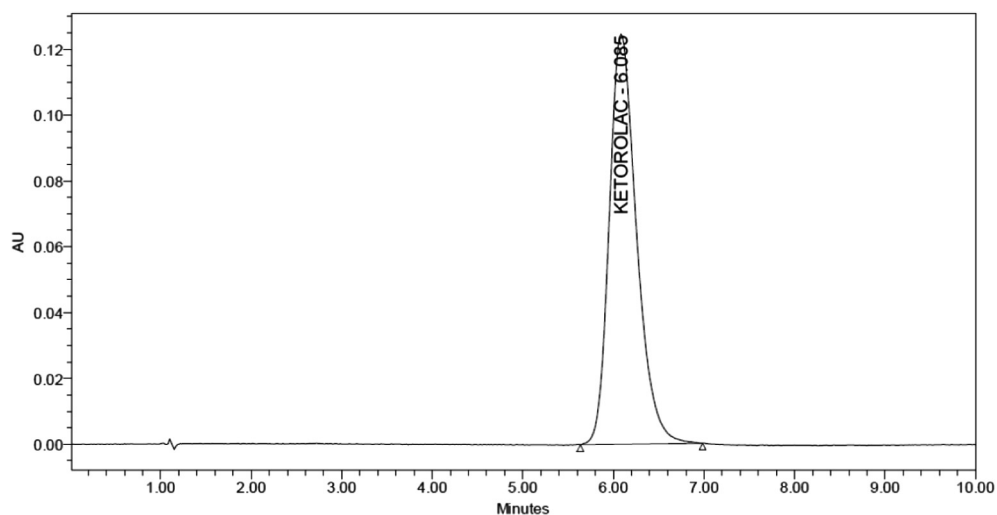**Figure 8** Intermediate precision chromatogram.

Table 3 Precision study of the method.

S. no	Sample area	% Assay	Amount present (mg)
1	2606386	100.4	0.47
2	2698061	103.9	0.48
3	2530365	97.47	0.43
4	2591486	99.82	0.46
5	257270	99.19	0.45
6	2576739	99.25	0.46
MEAN		99.99	0.45
SD		56171.2	
% RSD		1.16	

linear over the concentration range studied for Ketorolac Tromethamine with a correlation coefficient (r) of 0.999.

4.4. Stability of sample solution

The sample solution was prepared as per the test method, and analyzed initially at different time intervals by keeping the solution at room temperature. The percentage response between the initial and different time intervals shows that the sample solutions were stable for at least 24 h at room temperature (Table 4).

4.5. Robustness

Robustness of the method was determined by analyzing the standard solution at normal operating conditions by changing

Table 4 Stability study.

S. no	Time interval (h)	Peak response
1	0	2606836
2	2	2698061
3	6	2530365
4	10	2591486
5	16	2572701
6	24	2576739
Mean		2596031.33
SD		51277.56
% RSD		0.019

some operating analytical conditions such as flow rate, column, oven temperature, detection wavelength and the mobile phase. The conditions with variation and their results were shown in Table 5. The tailing factor is around unity indicative of peak symmetry and theoretical plate counts were also above 2000. Hence robustness of the extent of variations applied to analytical conditions was shown in Fig. 9.

4.6. Accuracy

The percentage recovery experiments were performed by adding a known quantity of pure standard drug into the pre-analyzed sample. The solution equivalent to 100 mg of Ketorolac Tromethamine was accurately weighed into a 100 mL volumetric flask. The sample was then spiked with standard at level 50%, 100% and 150% of test concentration. The resulting spiked sample solutions were assayed in triplicate and the results were compared and expressed as percentage. The mean percentage recovery of Ketorolac Tromethamine was found to be in the range between 98.54 and 102.5 which are within the acceptance limits as shown in Table 6 and Fig. 10.

4.7. Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected, but not necessarily quantified, under the stated experimental conditions. LOD and LOQ were calculated by using standard deviation and slope values obtained from the calibration curve by using the formula LOD = 3.3 (SD/S) and LOQ = 10 (SD/S). The LOD and LOQ values for Ketorolac Tromethamine were found to be 0.116 $\mu g\ mL^{-1}$ and 0.32 $\mu g\ mL^{-1}$ respectively as shown in Figs. 11 and 12 and Table 7.

5. Results and discussion

A different combination of mobile phases and chromatographic conditions were tried and a mobile phase containing ammonium dihydrogen phosphate and methanol (44:55 V/V), Kromosil C_{18} (150 cm × 4.6 mm × 5 μ) column, 1.5 mL min^{-1} flow rate, 10 μL injection volume, 30 °C column oven temperature, 314 nm wavelength and 20 min run time was found to be

Table 5 Robustness studies.

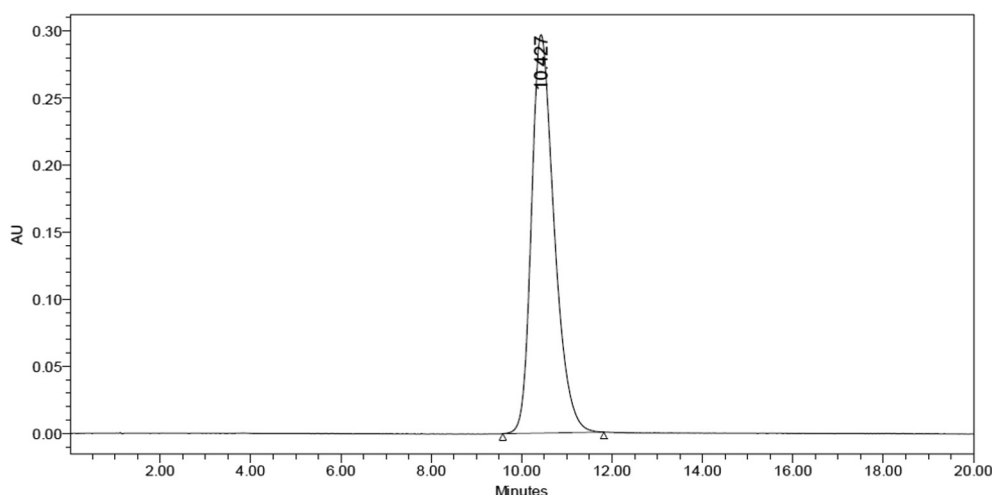| System suitability parameters (variations)		% RSD of peak area response (n = 3)	Mean tailing factor (n = 3)	Mean retention time in min (n = 3)
Change in (%)	+5	1.20	0.67	6.01
Organic phase	−5	1.23	0.86	6.05
Change in pH	+3	0.019	1.28	6.06
	−3	0.017	1.30	6.08
Change in flow	+1.5	0.004	1.22	6.75
	−1.5	0.003	1.25	6.85
Change in temp.	+25	0.003	1.19	5.18

Figure 9 Robustness chromatogram.

Table 6 Accuracy study.

Spike level (%)	Amount added in (μg mL⁻¹)	Peak area	% Recovery	% Mean recovery
50	0.12	1347374	103.90	102.5
50		1308694	100.83	
50		1336027	102.93	
100	0.25	2635598	101.54	99.44
100		2575802	99.32	
100		2530358	97.48	
150	0.36	3532611	96.23	98.54
150		3858796	99.10	
150		3906218	100.31	
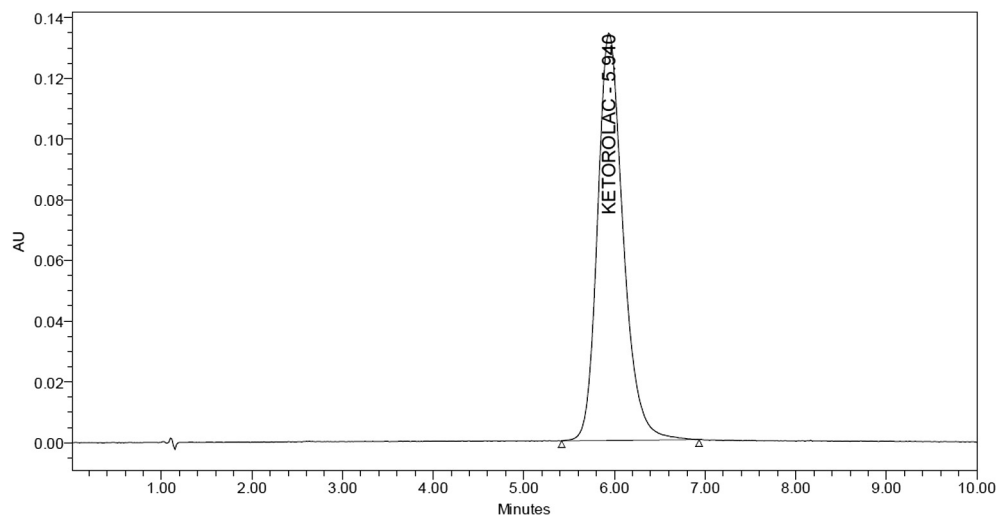
Figure 10 Accuracy chromatogram.
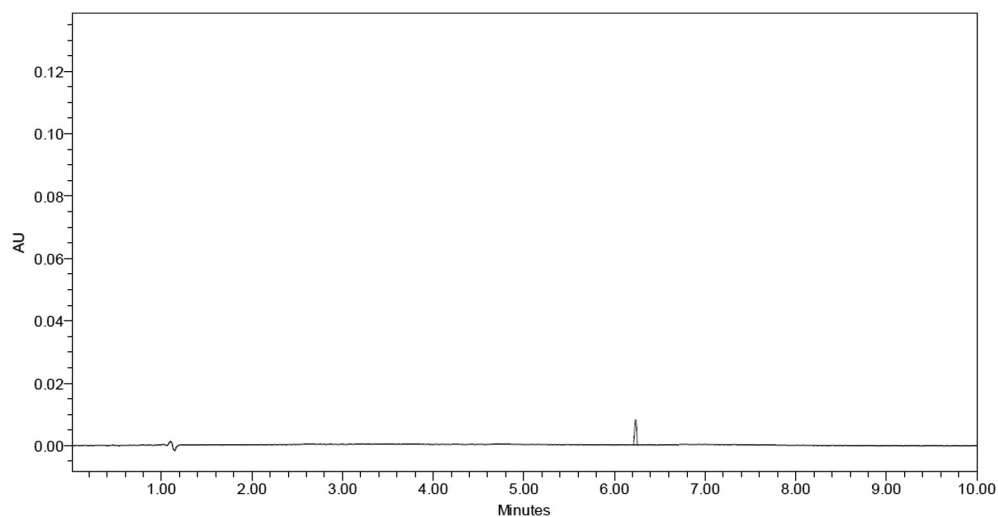
Figure 11 LOD.

suitable for all combinations. These chromatographic conditions gave retention time of 6.01 min. Specificity of the method was checked by injecting the placebo solution, no peaks were found at the retention time of Ketorolac Tromethamine. The stability of the sample solution was evaluated by preparing a sample solution as per the proposed method and analyzed initially and at 1 h intervals up to 24 h by keeping the sample solution at room temperature. The results of the stability studies showed that the solution of the drug was found to be stable for 24 h at room temperature. System precision and method precision results showed the % RSD of 1.16 and 0.02, respectively. A good linearity relationship indicated by correlation
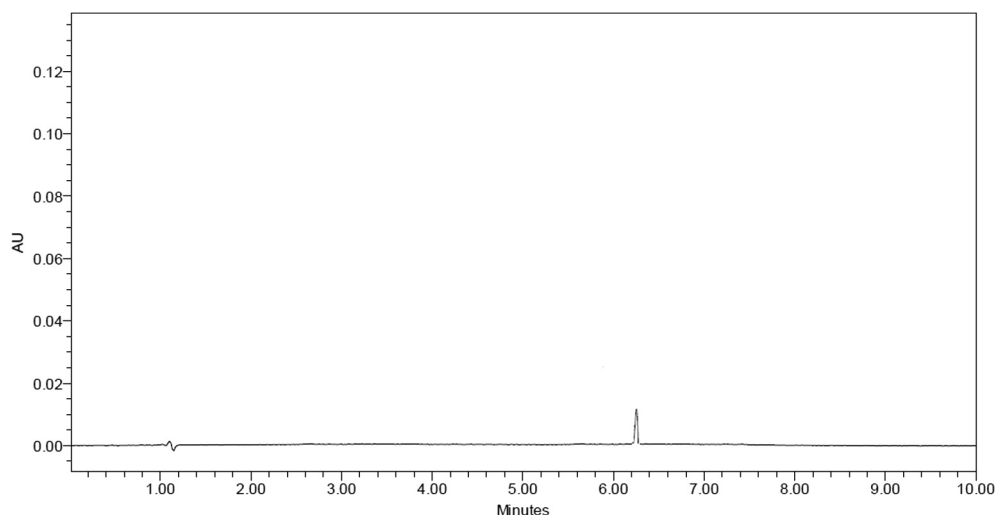
Figure 12   LOQ.

Table 7   LOD and LOQ.

S. no	Concentration (µg mL^{-1})	Peak area
1	50	1341056
2	70	1814323
3	100	2582490
4	120	3087955
5	150	3877114

coefficient (r) value 0.999 was observed between the concentrations of 50–150 µg mL^{-1} of Ketorolac Tromethamine. Intermediate Precision was done by changing the analyst, column, with the same chromatographic conditions and the obtained results were within the limits. The Robustness method was evaluated by deliberately varying the chromatographic conditions of the method such as mobile phase methanol content, flow rate, column temperature and wavelength. The parameters like tailing factor and retention time showed adherence to the limits. The accuracy of the method was determined and the percentage recovery was calculated. The data indicate an average of 102.5% recovery of the standard sample.

6. Conclusion

The method developed for Ketorolac Tromethamine was found to be a simple process and the procedure does not involve any experimental conditions. The validation results indicated that the method was specific, accurate, linear, precise, rugged and robust. The runtime was relatively 20 min which enabled a rapid quantification of many samples in routine and quality control analysis of ophthalmic formulation.

Acknowledgement

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