RESEARCH ARTICLE

Method Development and Validation of a New Stability-indicating Liquid Chromatographic Method for the Quantification of Ketorolac Tromethamine (A Nonsteroidal Anti-inflammatory Drug)

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Abstract

Introduction: A new liquid chromatographic method was established for the determination of ketorolac tromethamine (KTM). KTM is a nonsteroidal anti-inflammatory drug. **Materials and Methods:** Shimadzu Model CBM-20A/20 Alite with C8 Phenomex column (250 mm × 4.6 mm i.d., 5 µm particle size) was used for the chromatographic study. A mixture of 0.1 M ammonium formate and methanol was chosen as mobile phase with flow rate of 0.8 mL/min (UV detection at 241 nm). KTM was subjected to acidic, alkaline, oxidation, and thermal stress degradations. **Results and Discussion:** The method was validated as per ICH guidelines, and the regression equation was found to be y = 68207x +57590 (R^2 = 0.9997). The limit of quantification and limit of detection were found to be 0.7834 and 0.2571 µg/mL, respectively. KTM is found to be highly resistant toward all degradations. **Conclusions:** The method was reported to be robust and specific and can be applied for the assay of pharmaceutical formulations.

Key words: Ketorolac tromethamine, reversed-phase high-performance liquid chromatography, stability indicating, validation

INTRODUCTION

Ketorolac tromethamine [Figure 1][1] acts by inhibiting the bodily synthesis of prostaglandins. An ophthalmic solution of ketorolac is available and is used to treat eye pain and to relieve the itchiness and burning of seasonal allergies. Literature survey reveals that liquid chromatographic methods have been developed for the determination of ketorolac tromethamine (KTM) in pharmaceutical formulations[2-7] as well as in human plasma.[8] The authors have proposed a new liquid chromatographic method for the determination of KTM in pharmaceutical dosage forms.

MATERIALS AND METHODS

Chemicals and reagents

Methanol, sodium hydroxide, hydrochloric acid, ammonium formate, and hydrogen peroxide (H_2O_2) were purchased from Merck (India). All chemicals are of HPLC grade. All chemicals were of analytical grade and used as received. KTM is available with brand names Toradol, Acular, and Sprix.

Instrumentation

Chromatographic separation was achieved using Shimadzu Model CBM-20A/20 Alite HPLC system, equipped with SPD M20A prominence photodiode array detector with C8 Phenomenex column (250 mm × 4.6 mm i.d., 5 µm particle size) maintained at 25°C.

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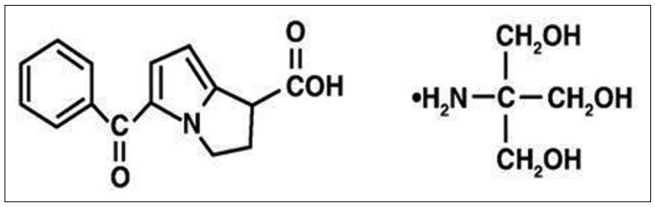

Figure 1: Chemical structure of ketorolac tromethamine

Chromatographic conditions

Isocratic elution was performed using 0.1 M ammonium formate and methanol (20:80%, v/v) and the flow rate was 0.8 mL/min. The overall run time was 10 min. The detection was carried at 241 nm. 20 µL of sample was injected into the HPLC system, and all chromatographic conditions were performed at room temperature (25°C±2°C).

Preparation of 0.1 M ammonium formate

0.1 M ammonium formate was prepared by weighing accurately 7 g of ammonium formate into a 1000 mL volumetric flask and diluted with HPLC-grade water. The resulting solution was sonicated for half an hour and filtered.

Preparation of stock solution

The stock solution was prepared by transferring accurately 25 mg of KTM into a 25 mL volumetric flask and diluting with mobile phase (1000 µg/mL), and further dilutions were made on daily basis from the stock solution with mobile phase as per the requirement and filtered through 0.45 µm membrane filter before injection.

Method validation

A series of solutions (1–150 µg/mL) were prepared from the KTM stock solution and 20 µL of each solution was injected into the HPLC system, and the peak area of the chromatogram was noted. Calibration curve was plotted by taking the concentration of the solutions on the x-axis and the corresponding peak area values on the y-axis. The intraday and interday precision studies were evaluated at three different concentration levels (10, 50, and 100 µg/mL) (n = 3) on the same day and on three different days, i.e. day 1, day 2, and day 3 and the percentage RSD was calculated. The accuracy of the assay method was evaluated using standard addition and recovery experiments (80, 100, and 120%). The robustness of the assay method was calculated by introducing small changes in the chromatographic conditions which includes detection wavelength (239 and 243 nm), mobile phase composition (78 and 82%), and flow rate (0.7 and 0.9 mL/min).

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

The LOQ and LOD were based on the standard deviation of the response and the slope of the constructed calibration curve (n=3), as described in ICH guidelines.[9] Sensitivity of the method was established with respect to LOD and LOQ for analytes.

Forced degradation studies

Forced degradation studies were performed to evaluate the stability-indicating properties and specificity of the method.[10-13] All solutions for stress studies were prepared at an initial concentration of 50 µg/mL of KTM and refluxed for 60 min at 80°C and then diluted with mobile phase.

Acidic degradation was performed by treating the drug solution (50 µg/mL) with 0.1 N HCl for 60 min in a thermostat maintained at 80°C. The stressed sample was cooled, neutralized with NaOH, and then diluted with mobile phase as per the requirement. 20 µL of this solution was injected into the HPLC system.

Alkaline degradation was performed by treating the drug solution (50 µg/mL) with 0.1 N sodium hydroxide for 60 min in a thermostat maintained at 80°C. The stressed sample was cooled, neutralized with HCl, and then diluted with mobile phase as per the requirement, and 20 µL of the solution was injected into the HPLC system.

Oxidation degradation was performed by treating the drug solution (50 µg/mL) with 30% H_2O_2 for 60 min in a thermostat maintained at 80°C. The drug solution mixture was cooled and then diluted with mobile phase as per the requirement, and 20 µL of the solution was injected into the HPLC system.

Assay of Ketorolac tromethamine eye drops

The available brand was procured from the local pharmacy store, and the contents were transferred carefully into the volumetric flasks and extracted with mobile phase after sonication for 30 min. The contents were filtered, diluted with mobile phase and 20 µL of these solutions were injected into the HPLC system after filtering through 0.45 µm membrane. The peak area was noted from the respective chromatogram, and the percentage purity was calculated from the linear regression equation.

RESULTS AND DISCUSSION

Method development and optimization

A new liquid chromatographic method has been proposed for the determination of KTM in pharmaceutical dosage

forms. Initially, the drug solution was analyzed using a mixture of 0.1 M ammonium formate:methanol (40:60% v/v) with a flow rate of 0.8 mL/min, but the peak was obtained at R_t 16.05 min as well as the peak symmetry was also not satisfactory. Therefore, the mobile phase ratio has been changed as 30:70% v/v and the drug sample was injected where a sharp peak was eluted at 7.96 min with tailing (>2). Then, the mobile phase composition was modified as 20:80% v/v with the same flow rate, and the drug was eluted at 4.048 ± 0.03 min (UV detection at 241 nm).

Method validation

KTM has shown linearity over a concentration range of 1–150 µg/mL [Table 1] with percentage RSD 0.27–0.90 and the chromatographic response was shown in Figure 2. The linear regression equations were found to be y = 68207x +57590 (R^2 = 0.9997). The LOQ and LOD were found to be 0.7834 and 0.2571 µg/mL, respectively.

The accuracy was studied by the recovery test (80, 100, and 120%). A known concentration of KTM formulation solution (5 µg/mL) was added to pure drug (API) solutions to yield the total concentration of 9, 10, and 11 µg/mL [Table 2] and thereby the percentage recovery was calculated (98.20–99.27%). The intraday precision of the method was determined by assaying three samples of each at three different concentration levels (10, 50, and 100 µg/mL) on the same day. The interday precision was calculated by assaying three samples of each at three different concentration levels (10, 50, and 100 µg/mL) on three different days. The percentage RSD for intraday precision was found to be 0.25–0.45, whereas the interday precision was found to be 0.82–1.05 [Table 2]. The percentage RSD was 0.10–1.84 for KTM (<2.0%) indicating that the proposed method is robust [Table 3].

Assay of KTM eye drops

The proposed method was applied for the determination of KTM in marketed formulations, and the percentage recovery was found to be 99.3–99.7 [Table 4]. The chromatogram of the mobile phase (blank) and the drug peak was shown in Figure 3a and b.

Stress degradation studies

Very slight decomposition (<5%) was observed in all the degradation conditions, i.e. acidic (0.56), alkaline (1.00%), thermal (0.48%), and oxidation (1.29%) reporting that KTM is highly resistant toward all the applied stressed conditions

Table 1: Linearity of ketorolac tromethamine

Concentration (µg/mL)	*Mean peak area±SD	RSD (%)
1	38585±135.0475	0.35
5	178041±801.18	0.45
10	394646±2052.15	0.52
20	826532±2231.63	0.27
50	1762744±12691.7	0.72
100	3572934±29298.0	0.82
150	5292390±47631.5	0.90

*Mean of three replicates

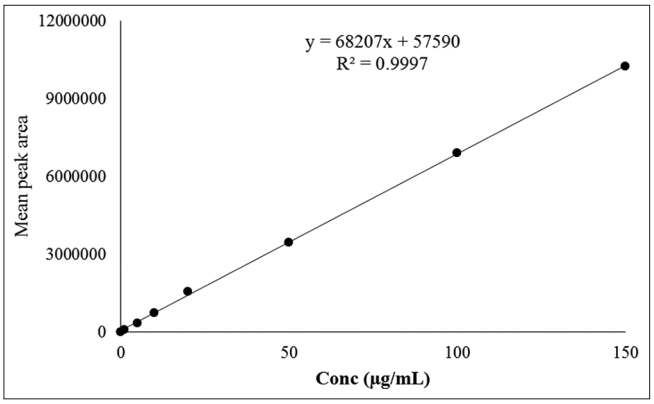

Figure 2: Calibration curve of ketorolac tromethamine

Table 2: Precision and accuracy studies of ketorolac tromethamine

Concentration (µg/mL)	Intraday precision	Interday precision
	*Mean peak area±SD (%RSD)	*Mean peak area±SD (%RSD)
10	394654±986.63 (0.25)	397425±3258.8 (0.82)
50	1765487±6355.7 (0.36)	1785139±16780.3 (0.94)
100	3524751±15861.3 (0.45)	3578215±37571.2 (1.05)

Accuracy

Spiked concentration (µg/mL)	Total concentration (µg/mL)	*Mean peak area±SD (% RSD)	Drug Found (µg/mL)	% recovery
4 (80%)	9	355248±639.44 (0.18)	8.87	98.5
5 (100%)	10	394857±947.65 (0.24)	9.82	98.2
6 (120%)	11	431547±1251.48 (0.29)	10.92	99.27

*Mean of three replicates

Table 3: Robustness study of ketorolac tromethamine

Parameter	Condition	*Mean peak area	*Mean peak area±SD (% RSD)
Flow rate (±0.1 mL/min)	0.7	395321	394857±402.38 (0.10)
	0.8	394646	
	0.9	394604	
Detection wavelength (±2 nm)	239	382147	391182.3±7795.49 (1.84)
	241	394646	
	243	396754	
Mobile phase composition (0.1 M ammonium formate: methanol) (±2, v/v)	18:82	397524	394474.7±3138.50 (0.79)
	20:80	394646	
	22:78	391254	

*Mean of three replicates

Table 4: Analysis of ketorolac tromethamine in ophthalmic formulation

Formulation	Labeled claim (%)	Amount found* (%)	Recovery* (%)
Brand I	0.1	0.0993	99.3
Brand II	0.1	0.0997	99.7

*Mean of three replicates

Table 5: Stress degradation studies of KTM

Stress conditions	*Mean peak area	*Drug recovered (%)	*Drug decomposed (%)	Theoretical plates	Tailing factor
Standard drug (untreated)	1762744	100	-	9414.140	1.162
Acidic degradation	1752911	99.44	0.56	5620.746	1.034
Alkaline degradation	1745142	99.0	1.00	8125.784	1.044
Oxidative degradation	1740158	98.71	1.29	8441.783	1.187
Thermal degradation	1754284	99.52	0.48	7603.652	1.183

*Mean of three replicates. KTM: Ketorolac tromethamine

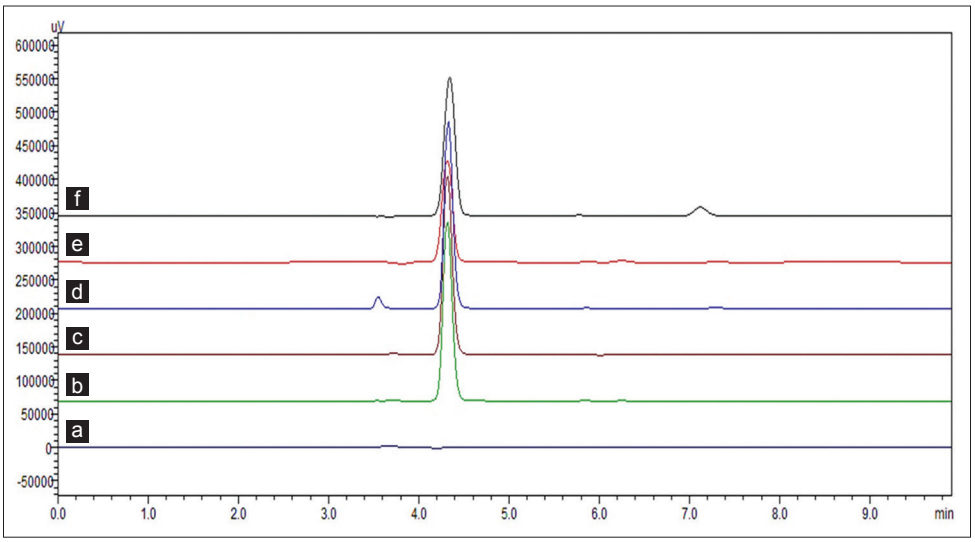

Figure 3: Typical chromatograms of ketorolac tromethamine (a) blank (b) standard (50 µg/mL) (c) thermal (d) oxidation (e) alkaline (f) acidic degradations

[Table 5]. Typical chromatograms obtained following the assay of stressed samples are shown in Figure 3a-f. The 3D chromatograms of the stressed samples were shown in Figure 4.

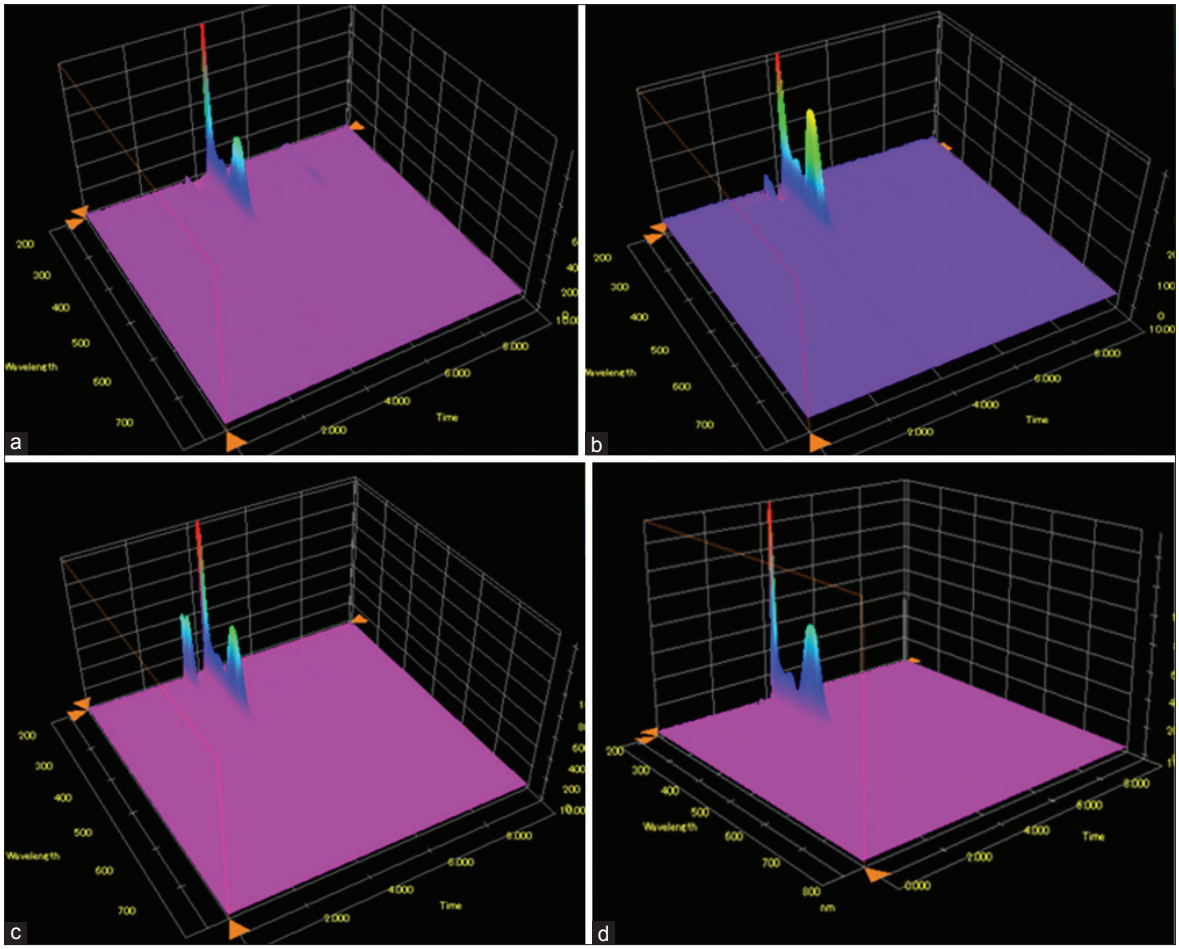

Figure 4: 3D chromatograms of ketorolac tromethamine (a) acidic (b) alkaline (c) oxidation (d) thermal degradations

CONCLUSION

The proposed stability-indicating HPLC method was validated as per ICH guidelines and applied for the determination of Ketorolac tromethamine in pharmaceutical dosage forms and can be successfully applied to perform long-term and accelerated stability studies of Ketorolac tromethamine formulations. It was observed that Ketorolac tromethamine is more stable during the forced degradation studies.

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