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Stability-Indicating RP-HPLC Method Development and Validation for the Quantification of Ketorolac Tromethamine Tablets in Solid Oral Dosage Forms

Naveen Maddukuri[1], Ashok Morsu[2], Teja Kamireddy[3], Prasanna Kumar Lankalapalli[4], Pranitha Sambu[5], Vijaykumar chollety[6]*

[1]Invagen Pharmaceutical Inc, 550S Research Pl, Central lslip, NY, USA-11722
[2]Thermo Fisher 3900 paramount parkway Morrisville N.C.27560
[3]August Bioservices 1845 Elm Hill Pike, Nashville, TN, 37210
[4]Visual Technologies LLC, 5851 Legacy Circle, Plano TX 75024
[5]Aurex Laboratories Pvt Ltd. Prashanthi nagar, Kukatpally, Hyderabad, India
[6]*Ascent pharmaceuticals inc ,400 S Technology Dr, Central lslip, NY,11722
cholletivijay@gmail.com

Abstract
The present study discusses the development of a simple, rapid, specific, precise, and accurate stability-indicating HPLC method for the analysis of Ketorolac tromethamine tablet dosage forms. Chromatographic separation was achieved using a mobile phase comprising methanol, water, and glacial acetic acid in a ratio of 550:440:10 (v/v/v). Detection was carried out at 254 nm for Ketorolac tromethamine. Analytical techniques were optimized to enhance sensitivity and specificity for the estimation of pharmaceutical drug products. Stress studies were evaluated under different ICH conditions. The optimized HPLC method was validated in accordance with current ICH guidelines, demonstrating high specificity with linearity in the range of 2.5μg/mL-50μg/mL for Ketorolac tromethamine, and a correlation coefficient >0.999. The method exhibited accuracy exceeding 97%. In stress studies, Ketorolac tromethamine was found to be sensitive to acidic and oxidative stress conditions. The method was deemed suitable for quality control of Ketorolac tromethamine tablets as well as for stability-indicating studies.

Keywords: Ketorolac tromethamine (KTR), HPLC, Method development, Validation, PDA

1. Introduction

KTR is non-steroidal anti-inflammatory drugs (NSAIDs) are prescribed to treat pain and inflammation caused by musculoskeletal and joint problems, as well as surgical procedures. Due to the high prevalence of rheumatoid illnesses in India, these medications are widely used. Many NSAIDs have been developed, one of which is KTR (Figure 1). Chemically, KTR is (±)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid, 2-amino-2-(hydroxymethyl)-1,3-propanediol. It is a potent antipyretic and anti-inflammatory agent, being 800 times more potent than aspirin. A literature survey reveals several methods reported for the determination of Ketorolac Tromethamine, including high-performance liquid chromatography (HPLC) [1],[3],[4],[5], HPTLC [6], and UV-visible spectroscopy [2]. The initial literature search indicated that most of the reported HPLC methods for

Ketorolac Tromethamine, including those in official compendia, were developed using C8, C18, or polymeric columns. However, these methods often exhibited poor peak shapes and considerable tailing. Consequently, efforts were made to develop a simple method according to ICH guidelines using a C18 column, with a focus on optimizing conditions such as lowering the column temperature. Therefore, forced decomposition studies were carried out in compliance with ICH requirements, and the method was developed and validated to the best of our understanding.

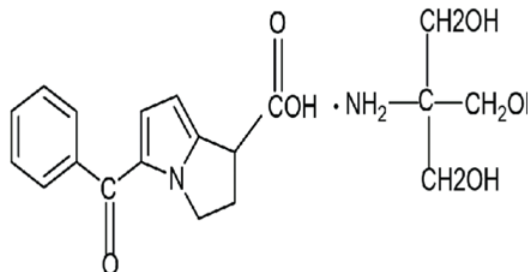

Figure 1: Structure of Ketorolac tromethamine

The current study is the first-ever report of an RP-HPLC stability-indicating method for Ketorolac Tromethamine in a solid formulation. Furthermore, forced degradation studies were conducted under various stress conditions, with no interference observed from unknown peaks or inactive excipients in the solid dosage formulation.

2. Materials and Methods

Chemical and reagents

The AR grade Glacial Acetic acid was procured from VWR chemicals, USA. The HPLC grade of Methanol (J.T. beaker) with certified purity of 99.9% was purchased from Avantor performance materials, LLC, Radnor, PA, USA. High quality In-House purity water was used for the experiments (TOC <500ppb, pH about 7.0, Conductivity < 1.0 µs/cm, finally exposed to UV radiation and followed filtered through 0.2 µm filter). KTR was procured from Aurore laboratories private limited, Hyderabad, India.

Instrumentation and Software

Waters HPLC system Alliance e2695 separation module with auto injector, temperature controller for sample storage and column was used for current analysis. The signal output was observed through Empower 3 Software Build 3471 SPs Installed: Feature Release 3 DB ID: 2639633283. The LC column is Hypersil BDS C18, 250 x 4.6 mm, 5 µm, is manufactured by Thermo fisher. Analytical balance model AX205 (make: Mettler Toledo), sonicator (make: ENERTECH), Rotary shaker (make: REMI; model: RS – 24BL) were employed in this work.

Preparation of diluent

Mix 500 mL of methanol, 500ml of Water in the ratio of 50:50 (v/v) and mixed well.

Preparation of standard solution

Preparation of standard stock solution

Accurately weighed and transferred about 20 mg of Ketorolac Tromethamine standard into a 100-mL volumetric flask. Add 50ml of methanol, sonicate to dissolved, dilute to volume with methanol, the solution was mixed well and label. The concentration was about 200µg/mL of KTR.

Preparation of standard solution (24µg/mL of Ketorolac Tromethamine)

Pipetted 3.0 mL of KTR Standard Stock Solution into a 25-mL volumetric flask, add dilute to the volume with diluent and the solution was mixed well. The concentration was about 24µg/mL of KTR.

Chromatographic conditions

Chromatographic separation was achieved using a mobile phase consisting of methanol, water, and glacial acetic acid in a ratio of 550:440:10 (v/v), with a flow rate of 1.2 mL/min. The diluent was prepared by mixing 500 mL of methanol and 500 mL of water in a 1:1 ratio (v/v). The LC column used was a Hypersil BDS C18 (250 x 4.6 mm,5

µm). The isocratic method was employed, with a column temperature of 30°C and a run time of 18 minutes. The injection volume was 50µL, and component detection was performed at 254nm.

Preparation of Sample stock (200 µg/mL of KTR)

Weighed and transferred 5 Tablets into a clean dry 250ml volumetric flask. Added 25ml of water and sonicate to disperse the tablets completely, **methanol** was added to about 60ml, and the solution was sonicate for about 20min with intermittent shaking, the flask was allowed to cool the room temperature, diluted to volume with **methanol** and mixed well. The solution was then Centrifuged at 5000RPM for approximately 5min, then filtered the solution through 0.45µm PVDF discarding the first 3 mL of filtrate.

Preparation of Sample (24 µg/mL of KTR)

Pipet 5.0 mL of Sample Stock Solution into a 50-mL volumetric flask. Diluted to volume with diluent and mixed well and label as Sample Solution. The concentration was about **24 µg/mL of KTR**.

3. Results and Discussion

Method Development and Optimization

The main aim of the current method was to separate Ketorolac Tromethamine in the presence of degradant impurities and the placebo in the sample. The selection of analytical conditions was based on the chemical nature of Ketorolac Tromethamine. A systematic study of various factors was undertaken by varying one parameter at a time while keeping all other conditions constant for the development of the analytical method. Ketorolac Tromethamine is soluble in polar solvents, and therefore, RP-HPLC was chosen. The selection of the stationary phase was based on factors such as back pressure, peak shape, theoretical plates, and day-to-day reproducibility of retention time for Ketorolac Tromethamine. After evaluating all these factors, the Hypersil C18 column (250 x 4.6 mm, 5 µm) was chosen for the analysis. The selection of the mobile phase was based on the chemical structure of the selected drug molecule. For the optimization of the mobile phase, preliminary trials were conducted under isocratic conditions using a mobile phase consisting of 2.72 g of monobasic potassium phosphate and methanol in a ratio of 55:45 (v/v) with a flow rate of 1.0 mL/min on an HPLC system equipped with a photodiode array (PDA) detector and a Thermo Fisher C18 column (150 x 4.6 mm, 5 µm). The peak was co-eluted with impurities and the main peak. To address this issue, an attempt was made using a mobile phase of 2.72 g of monobasic potassium phosphate (0.02 M) in 1000 mL of water, adjusted to pH 3.0 with orthophosphoric acid as the buffer, and mixed with methanol and acetonitrile in the ratio of 60:20:20 (v/v/v). However, the Ketorolac Tromethamine peak was still co-eluted with impurity peaks. Further optimization involved using a mobile phase consisting of 0.02 M phosphate buffer (pH 3.0 with orthophosphoric acid), methanol, and acetonitrile in a ratio of 50:30:20 (v/v/v), but the Ketorolac Tromethamine peak was still not separated from the impurity peak.

Another attempt was made using a mobile phase of methanol, water, and glacial acetic acid in a ratio of 55:44:1 (v/v/v) with a flow rate of 1.2 mL/min. Although there was some improvement, the separation and resolution of peaks were still unsatisfactory. Finally, the column was changed to a Thermo Fisher C18 column (250 x 4.6 mm, 5 µm) on an HPLC system equipped with a PDA detector. Under these conditions, the main peak was successfully separated from all impurity peaks. Upon optimizing the chromatographic conditions, the impurity and placebo peaks were well resolved from the KTR peak.

Based on these experiments, the optimized conditions (Fig. 2–Fig. 4) were determined. A Thermo Hypersil BDS C18 column (250 x 4.6 mm, 5 µm) was used as the stationary phase. The elution method was isocratic, with the column temperature maintained at 30°C, detection monitored at 254 nm, an injection volume of 50 µL, and a flow rate of 1.2 mL/min. The typical retention time of Ketorolac Tromethamine was about 10.2 min, respectively (see table 1).

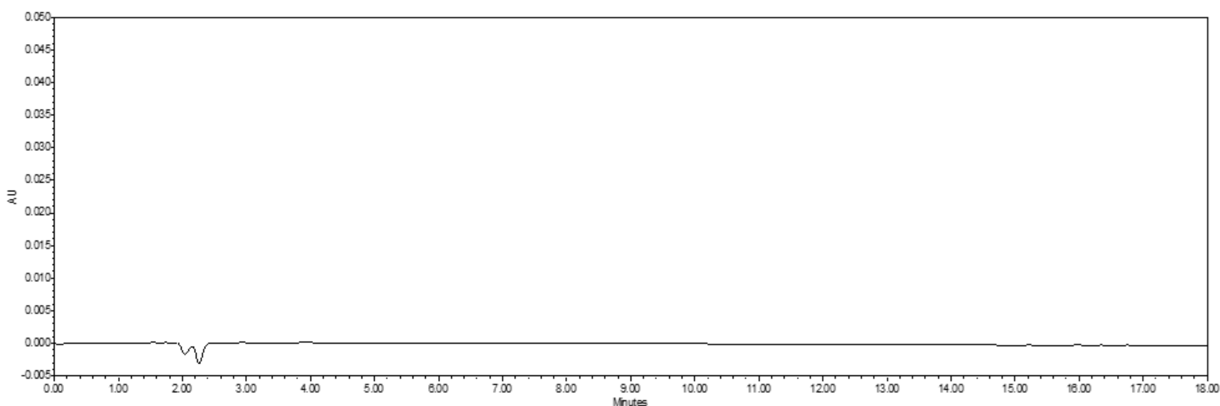

Figure 2: Typical Chromatogram of Blank

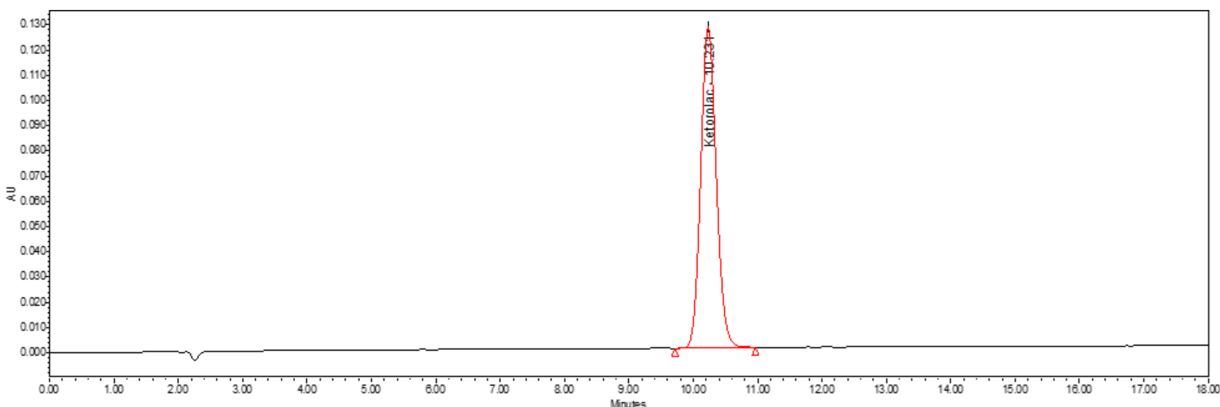

Figure 3: Typical Chromatogram of Standard solution

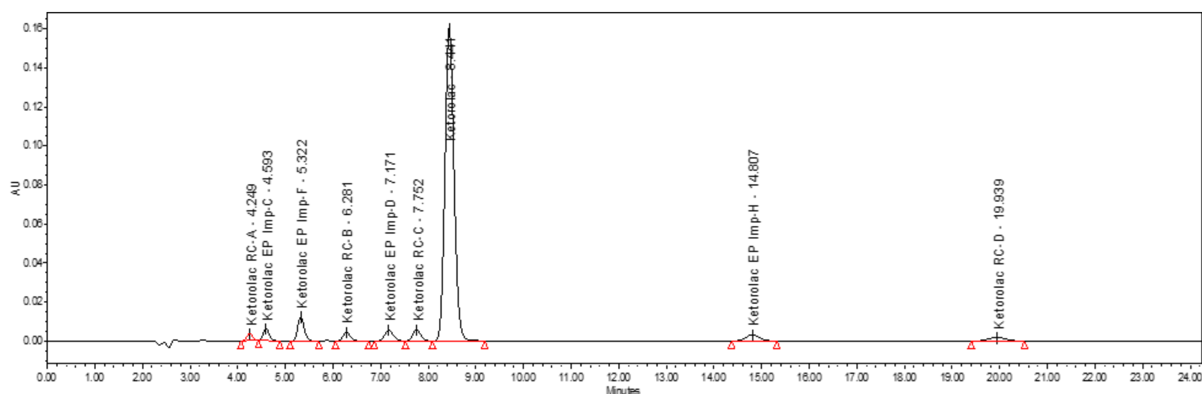

Figure 4: Typical Chromatogram of Spiked sample

Table 1: Optimized Method development conditions:

Mobile phase	**Column**	**Flow rate(ml/mint) elution mode column temp injection volume**	**Extraction solvent/diluent**	**Observation**	**result**
0.02M phosphate buffer and methanol in	Thermo fisher C18,	1.0ml/mint isocratic	Water and methanol in the	The KTR peak was co-eluted with	rejected

the ratio of (55:45) (v/v)	150 x 4.6 mm, 5µm	30°C,50µL	ratio of (50:50) (v/v)	impurities and the main peak	
0.02M phosphate buffer pH 3.0 and methanol and acetonitrile in the ratio of (60:20:20(v/v/v)	Thermo fisher C18, 150 x 4.6 mm, 5µm	1.0ml/mint Isocratic 30°C,20µL	Water and methanol in the ratio of (50:50) (v/v)	The KTR peak was still co-eluted with impurity peaks	Rejected
0.02M phosphate buffer pH 3.0 and methanol and acetonitrile in the ratio of (50:30:20(v/v/v)	Thermo fisher C18, 150 x 4.6 mm, 5µm.	1.0ml/mint isocratic 30°C,20µL	Water and methanol in the ratio of (50:50) (v/v)	The KTR peak was still not separated from the impurity peak	Rejected
Mixed methanol, water and glacial acetic acid in the ratio of 55:44:1(v/v/v)	Thermo fisher C18, 150 x 4.6 mm, 5µm	1.0ml/mint isocratic 30°C,20µL	Water and methanol in the ratio of (50:50) (v/v)	All peaks were separated from ketorolac peak but resolution was not satisfied.	Rejected
Mixed methanol, water and glacial acetic acid in the ratio of 55:44:1(v/v/v)	Thermo fisher C18, 250 x 4.6 mm, 5µm	1.2ml/mint isocratic 30°C,20µL	Water and methanol in the ratio of (50:50) (v/v)	All peaks were separated from ketorolac peak but resolution was satisfied.	Approved

Method Validation

The method was validated based on International Conference on Harmonization (ICH) Q2(R1)
Guidelines (ICH 2005). (Sreenivas, P. et al., 2022, Pippala et al., 2023,). Validation parameters included linearity, precision, accuracy, specificity and forced degradation (Jyothsna et al., 2024a, 2024b, Teja Kami Reddy et al., 2024)

Linearity

To prove the linearity of the optimized method, A sequence of concentrations were made for Ketorolac Tromethamine (2.4µg /mL to 48µg /mL) from the concentration range (10% to 200% with respect to standard concentration) by using suitable amount of the stock solutions. A curve was created by mapping the peak response and concentration. The component was shown correlation coefficient > 0.999.

The results of were shown Table 2.

Precision

Precision reflects the degree of agreement between individual test results obtained by applying the procedure or method to a homogeneous sample (Sreenivas et al., 2024a; Prasanna Lankalapalli et al., 2024). Typically, it is expressed as variance or standard deviation (SD). Under normal conditions, it serves as a measure of repeatability or reproducibility.

Intra-day precision was assessed under similar conditions by performing replicate applications and measuring peak areas six times on the same day. Inter-day precision was evaluated by repeating the assay six times over two different days, from which %RSD values were calculated. The % relative standard deviation was determined to be not more than 2.0. The intra-day and inter-day precision results are shown in Table 2 respectively.

Accuracy

To prove the accuracy of the optimized method, three levels (50%, 100%, and 150%) were prepared. Each sample was prepared in triplicate, with concentrations ranging from 12µg/mL, 24µg/mL, and 48µg/mL of KTR. The recovery was calculated as the ratio of the amount estimated to the amount spiked. All the method validation parameters results were shown in Table 2.

Table 2: Method validation summary for Ketorolac Tromethamine

Validation parameters	Ketorolac Tromethamine
Tailing factor	1.1
%RSD n=5	0.1
Linearity range(µg/ml)	2.52-48.12
Coefficient(r^2)	0.99999
Slope	80380.28
(%) Y-intercept	3259.90
Bias	0.0672
Residual sum of squares	101958552.4
ACC-50%mean, %RSD(n=3)	99.2, 1.3
ACC-100% mean %RSD(n=3)	100.8 ,0.8
ACC-150% mean %RSD(n=3)	101.0 ,0.5
Method precision %RSD(n=6)	1.0
Intermediate precision % RSD (n=12)	0.8

Specificity

Prepared blank, placebo as per the optimized test procedure and verified the interference of excipients and diluent peaks at retention time of active peaks (Santhi Priya et al., 2024 and Vaishnavi 2024). The results showed no interference at active peaks. To prove the stability indicating power of optimized analytical method performed the forced degradation in various conditions like acid, base, peroxide, thermal and humidity conditions as per the current ICH Q2(R1) guidelines.

Exposed the samples to 5 mL of 0.1 N hydrochloric acid at 80°C for 24 hr on water bath. Base hydrolysis exposed samples to 5mL of 0.1 N Sodium hydroxide at 80°C for 24 hr on water bath. Further neutralized and prepared samples as per the test procedure. For Oxidative degradation, sample was treated with 5 mL of hydrogen peroxide (3%v/v), at RT for 24 hr. For thermal degradation, sample powder was exposed to 105°C for 7 days in hot air oven. For Humidity degradation, sample powder was exposed to 90% for 7 days on the desiccator. During the acid, Base, water, peroxide and thermal, humidity KTR were shown major degradation in acid and peroxide conditions. All chromatograms were shown no interference at retention time of Ketorolac Tromethamine and known impurities . The purity of peaks was monitored by using Empower 3 software, the purity angle is less than that of purity threshold in all stress conditions. All the conditions results were shown in Table 3.

Table 3: Forced degradation as per ICH for Ketorolac Tromethamine

Sample name	Condition	%Recovery Based on Control	Purity Angle	Purity Threshold
Control	Not Stressed	100.0	0.055	1.026
Acid Hydrolysis	5mL of 0.1N HCl at 80ºC for 24hours	95.2	0.690	1.035
Base Hydrolysis	5mL of 0.1N NaoH at 80ºC for 24hours	98.7	0.055	1.064
Water Hydrolysis	5mL of Water 80ºC for 24hours	100.0	0.057	1.020
Oxidation	5mL of 3%H_2O_2 RT for 24 hours	90.5	0.067	1.027
Thermal	105ºC for 7 Days	99.1	0.062	1.023
Humidity	90% for 7 Days	99.5	0.042	0.94

4. Conclusion

A simple, economical, rapid and RP—HPLC stability indicating method has been effectively optimized for. The optimized method was further validated for specificity, linearity, precision and accuracy, parameters. Further, stress studies were executed under various ICH stress conditions and proved stability indicating nature. Explicitly, the method was developed and validated and in quality control lab for stability analysis.

Conflict of interest

The authors declare no conflict of interest

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