Development And Validation of a Simple and Precise Hplc Method for The Estimation of Ketorolac Tromethamine and Its Application in Matrix Tablet Formulation

Pratiksha R. Meshram[1], Ajinkya V. Gulhane[2], Aachal S. Chimkar[3], Dr. Anil P. Dewani[4], Dr. Manisha D. Kitukale[5], Dr. Anil V. Chandewar[6]
[1,2,3,4] *Assistant professor, P. Wadhwani College of Pharmacy, Yavatmal-445001*
[5,6] *Principal, P. Wadhwani College of Pharmacy, Yavatmal-445001*

***Abstract*—A simple, rapid, accurate, and precise isocratic high-performance liquid chromatography (HPLC) method was developed and validated for the quantitative estimation of Ketorolac Tromethamine (KT) in matrix formulations. The chromatographic separation was performed using a Kintex C8 column (250 mm × 4.6 mm, 5 µm) at room temperature with a mobile phase consisting of Methanol: Acetonitrile: MKP and MSP in the ratio of 5:15:80 (v/v), delivered at a flow rate of 1.0 mL/min. Detection was carried out using a photodiode array (PDA) detector at a wavelength of 319 nm. The retention time of KT was observed at 2.20 minutes. The developed method showed excellent linearity over a concentration range of 20% to 120%, with a correlation coefficient (R^2) of 0.9994. System suitability parameters and percentage assay results (ranging from 98% to 100%) were within acceptable limits. The percentage recovery for KT was found to be between 98% and 99.5%, confirming the method's accuracy. All validation parameters, including specificity, precision, linearity, accuracy, and robustness, complied with ICH guidelines. The results indicate that the developed HPLC method is reliable and suitable for routine analysis of Ketorolac Tromethamine in pharmaceutical matrix formulations.**

***Index Terms*—Ketorolac Tromethamine, HPLC, Method Development, Matrix Formulation, Assay, Recovery.**

I. INTRODUCTION

The development of reliable, accurate, and reproducible analytical methods is essential in pharmaceutical sciences to ensure the quality, efficacy, and safety of drug products. High-Performance Liquid Chromatography (HPLC) is one of the most widely used techniques due to its sensitivity, specificity, and suitability for analyzing active pharmaceutical ingredients (APIs) even in complex formulations.

This study focuses on developing and validating a simple, precise, and cost-effective HPLC method for the estimation of Ketorolac Tromethamine (KT), a potent NSAID used for short-term management of moderate to severe pain. The method was applied for the assay of KT in a matrix tablet formulation, which aims to provide controlled drug release, reduce dosing frequency, and enhance patient compliance—critical factors in managing chronic pain.

Ketorolac Tromethamine acts by inhibiting COX enzymes, reducing prostaglandin synthesis without central nervous system side effects typical of opioids. However, to mitigate its potential for adverse effects such as gastrointestinal or renal toxicity, controlled-release formulations like matrix tablets are beneficial.[1-14] Matrix tablets incorporate the drug within a polymeric matrix, using materials like HPMC or Carbopol, to achieve sustained release through diffusion or erosion mechanisms. Accurate drug quantification during formulation requires a validated analytical method.

This study aimed to (1) develop and validate a robust HPLC method in line with ICH Q2(R1) guidelines, and (2) apply it for KT assay in matrix tablets. The method was validated for parameters including linearity, accuracy, precision, specificity, robustness, and system suitability. By integrating method development with formulation design, this research

offers a standardized approach for KT estimation in modified-release systems, supporting quality control and contributing to enhanced therapeutic outcomes.

II. MATERIAL AND METHODS

Ketorolac Tromethamine (99% purity) was obtained from Yarrow Chem Laboratories, Mumbai, and used as the test drug. A marketed formulation, Ketorol-DT tablets (Dr. Reddy's Laboratories), was analyzed for comparison, using 10 tablets. HPLC-grade reagents acetonitrile, methanol, water, potassium dihydrogen phosphate, and sodium dihydrogen phosphate—were used for mobile phase and buffer preparation. Analysis was performed using an HPLC system with a UV detector. All equipment and glassware were analytical grade and pre-calibrated to ensure accuracy.

Analytical Method Development for Ketorolac Tromethamine by HPLC

A. Wavelength Selection: A 10 µg/mL solution of Ketorolac Tromethamine (KT) in methanol was scanned between 200–400 nm using a UV-Vis spectrophotometer. The maximum absorbance was observed at 319 nm, which was selected as the detection wavelength.

B. Chromatographic Conditions: Reverse-phase HPLC (RP-HPLC) was selected due to KT's polar nature and the technique's robustness and reproducibility for pharmaceutical compounds.

C. Mobile Phase Optimization: Various methanol-buffer mixtures were evaluated for retention time and peak shape. The ideal mobile phase yielded sharp, symmetrical peaks with minimal tailing.

D. Buffer Selection: Buffers with different pH and concentrations were tested. A pH of 4.8 using phosphate buffer gave the best peak characteristics and stability.

E. Stationary Phase: Three columns were assessed. The Kintex 5µ C8 (250 × 4.6 mm, 5 µm) column provided the best peak symmetry and resolution and was selected for further studies.

Mobile Phase Preparation: 1.4 g monopotassium phosphate and 1.2 g monosodium phosphate were dissolved in 500 mL water, sonicated, and pH adjusted to 4.8. The final mobile phase was composed of 5% methanol, 15% acetonitrile, and 80% phosphate buffer.

Diluent and Standard/Sample Preparation

Diluent: Methanol was used as the diluent for all standard and sample preparations.

Standard Stock Solution (100 µg/mL): 100 mg of Ketorolac Tromethamine was dissolved in methanol in a 1000 mL volumetric flask, sonicated, and volume adjusted. From this, 1 mL was diluted to 10 mL with methanol to obtain a 100 µg/mL working solution.

Sample Solution: Twenty Ketorol-DT tablets were weighed and powdered. A portion equivalent to 100 mg of KT (870.32 mg powder) was dissolved in methanol, sonicated, and made up to 1000 mL. The solution was filtered, centrifuged, and 1 mL was diluted to 10 mL to obtain the final sample solution.

Test Procedure: 20 µL of blank, standard, and sample solutions were injected into the HPLC system using a Kintex 5µ C8 column (250 × 4.6 mm, 5 µm). The mobile phase (methanol:acetonitrile:phosphate buffer, 5:15:80 v/v) was run isocratically at 1 mL/min, with detection at 319 nm. Peak areas were compared to calculate assay.

III. OPTIMIZED METHOD

SR. NO.	CHROMATOGRAPHIC CONDITION	
1.	Mobile phase	Methanol: ACN: MKP & MSP (0.02M)
2.	Concentration	05: 15: 80 % v/v
3.	pH	4.8
4.	Flow rate	1 ml/min
5.	Column	Kintex 5u C8 100A (250 cm x 4.6 mm, 5micron)
6.	Run time	10 min.

Buffer: Monopotassium phosphate (MKP) and Monosodium phosphate (MSP)

Method Validation (ICH Q2B Guidelines)

The analytical method was validated in accordance with ICH Q2B guidelines for parameters including system suitability, specificity, accuracy, linearity, precision, and robustness. System suitability was confirmed by injecting the KT standard five times, with all values within acceptable limits (%RSD ≤ 2.0%, tailing factor ≤ 2.0, and theoretical plates ≥ 2000). Specificity was established as no interference was observed at the retention time of KT in blank or placebo chromatograms, and both standard and sample had matching retention times. Accuracy was demonstrated using the standard addition method at 40%, 80%, 100%, and 120% levels, with mean recovery between 98.0% and 102.0%. Linearity was confirmed over a concentration range of 1–6 µg/mL with a correlation coefficient (r^2) ≥ 0.9990 and %RSD ≤ 2.0%. Precision was validated through intra-day repeatability, showing %RSD ≤ 2.0%. Robustness was assessed by altering the flow rate between 0.9 and 1.1 mL/min, with results consistently within acceptable limits, indicating the method's reliability.

Stock and Calibration Standards Preparation: 100 mg KT was dissolved in 1000 mL methanol. From this, solutions of 1–6 ppm were prepared by appropriate dilution in 100 mL volumetric flasks.

Matrix Tablet Preparation (Wet Granulation)

1. KT, ethyl cellulose, and excipients were weighed, sieved, and mixed.
2. Granules were formed using 5% PVP in alcohol.
3. Dried, sieved granules were lubricated with talc and magnesium stearate.
4. Final blend was compressed into tablets (8 mm round flat) using a 16-station rotary press.

Table 1: Composition of matrix tablet:

Sr. no.	Ingredient	F1	F2	F3
1.	Ketorolac tromethamine	0.2 gm	0.2 gm	0.2 gm
2.	Xanthin gum	0.2 gm	0	0.2 gm
3.	Guar gum	0	0.2 gm	0.2 gm
4.	Ethyl cellulose	0	0	0.2 gm
5.	Sod. alginate	0	0	0.2 gm
6.	Dicalcium phosphate	3.56 gm	3.56 gm	3.56 gm
7.	Magnesium stearate	0.04 gm	0.04 gm	0.04 gm

(Selected a batch F3 for formulation of matrix tablet.)

IV. RESULT AND DISCUSSIONS

System suitability

Sample solution of KT were injected three times into HPLC system as per test procedure. The system suitability parameters were evaluated from standard chromatograms obtained, by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from three replicate injections.

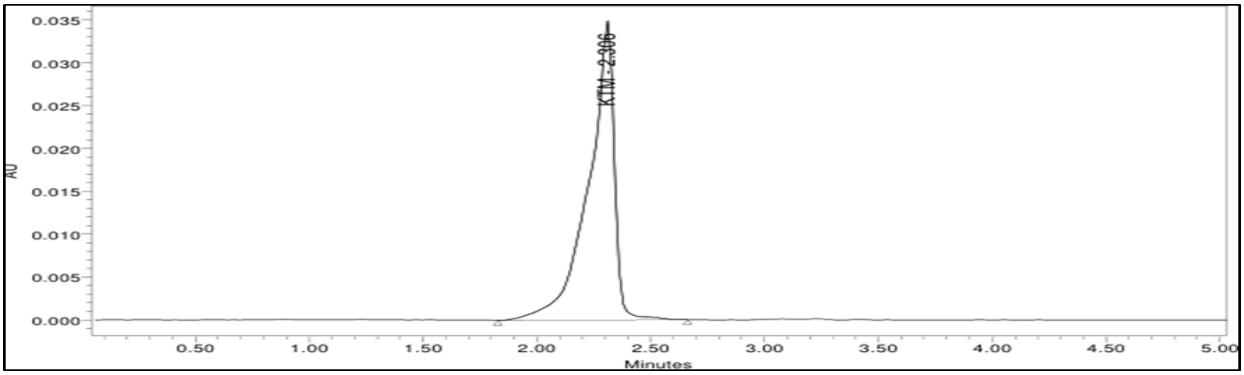

Figure 1: System suitability chromatogram for KT

Accuracy

Accuracy of an analytical method is the closeness of test result obtained by that method to the true value. The ICH document recommended that the accuracy should be assessed using a minimum of nine determinations over a minimum of three-concentration level, covering the specified range.

Accuracy studies

Accuracy for assay was performed at 4 levels, viz, 40%, 80%, 100% and 120 % of working concentration pre-analysed tablet solution (5 ppm) was spiked with standard at three concentration vise 40% (2ppm), 80% (4ppm), 100% (5 ppm), & 120 % (6 ppm). The standard spiked dilutions were analysed under developed chromatographic condition and amount of standard recovered was calculated and % recovery was reported.

Table 2: Accuracy study of KT by standard addition method at 40%, 80%, 100% and 120% levels

Sr No.	Level	Amount Added (ppm)	Mean Area ± %RSD (n=3)	Mean Area Recovered (n=3)	Amount Recovered (ppm) (n=3)	% Recovere d (n=3)
1.	40 %	2	15144 ± 1.08	14841	1.964	98.2
2.	80%	4	20056 ± 2.02	19654	3.92	98
3.	100%	5	23018 ± 2.03	22902	4.975	99.50
4.	120%	6	25555 ± 2.05	25299	5.946	99.1

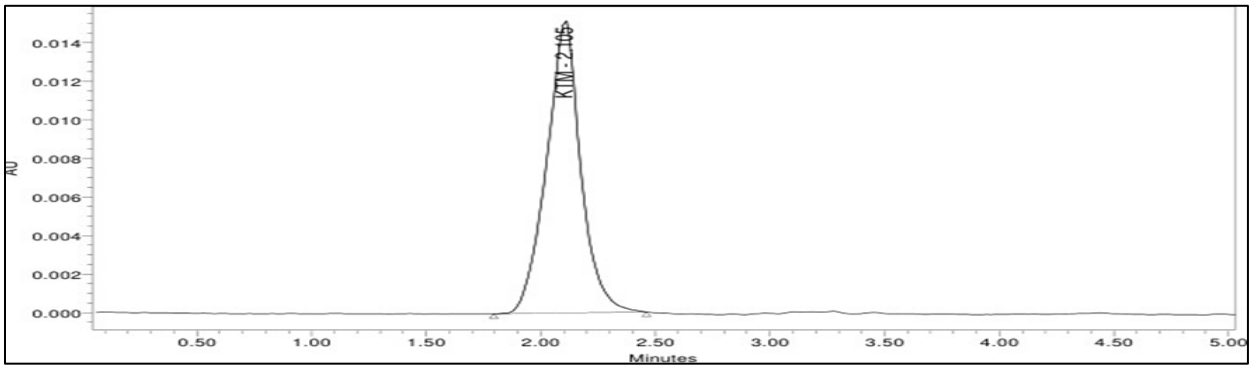

Figure 2: Accuracy chromatogram for KT

Linearity studies

Linearity study was performed at six concentration levels viz. 20%, 40%, 60%, 80%, 100%, and 120% of labelled claim. Each concentration was analysed by injecting 3 times under developed chromatographic condition. The results are presented in table. A plot of peak area against concentration was plotted, R^2 value and equation of regression was constructed.

Table 3: Linearity studies for KT

Sr. No.	Description	Concentration (ppm)	Mean Area ± %RSD (n=3)
1.	Linearity_20%	1	53892 ± 0.1
2.	Lineariry_40%	2	10820 0.33
3.	Lineariry_60%	3	15956 ± 0.69
4.	Lineariry_80%	4	20643 ± 0.29
5.	Lineariry_100%	5	26567 ± 1.04
6.	Lineariry_120%	6	31641 ± 0.98

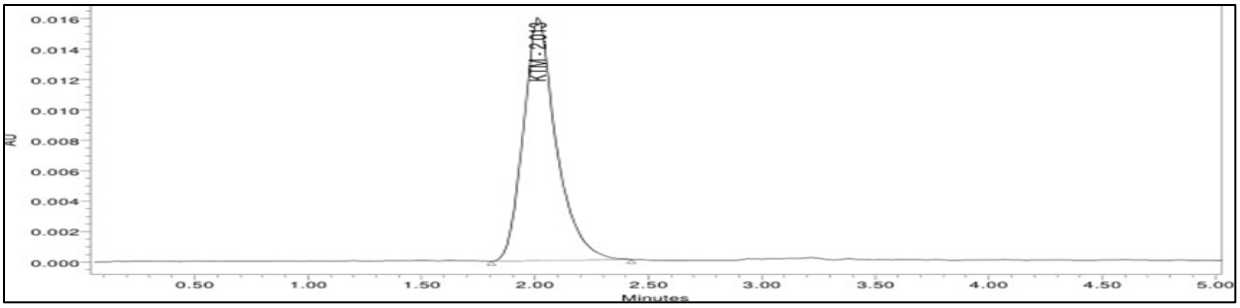

Figure 3: Linearity chromatogram of KT

Precision

Precision of analytical procedure expressed the closeness of agreement i.e., degree of scatter between a series of measurement obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision may be considered at three levels i.e., repeatability, intermediate precision and reproducibility.

Repeatability:

Repeatability expresses the precision under the same operating condition over a short interval of time repeatability is also termed as intra –assay precision.

Specificity:

The test was performed with blank solution, placebo, standard solutions were prepared and injected as described in the method of assay.

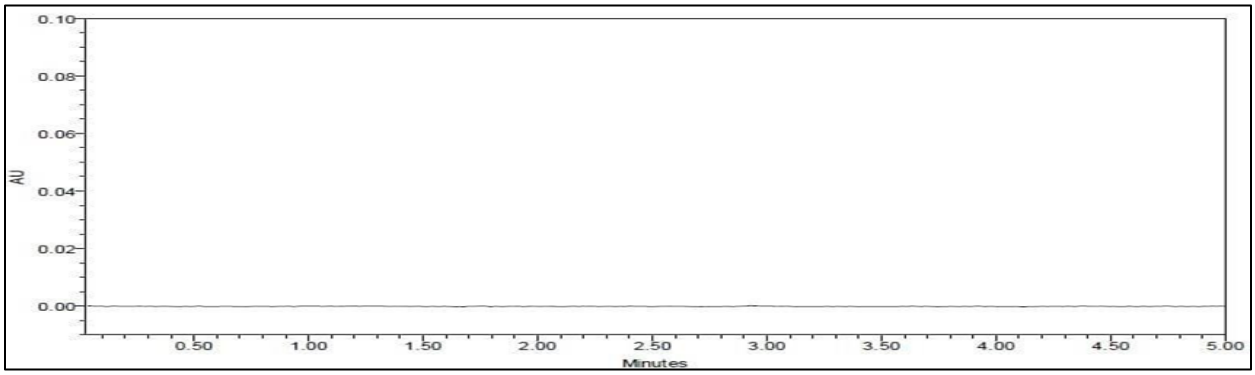

Figure 4: Blank chromatogram

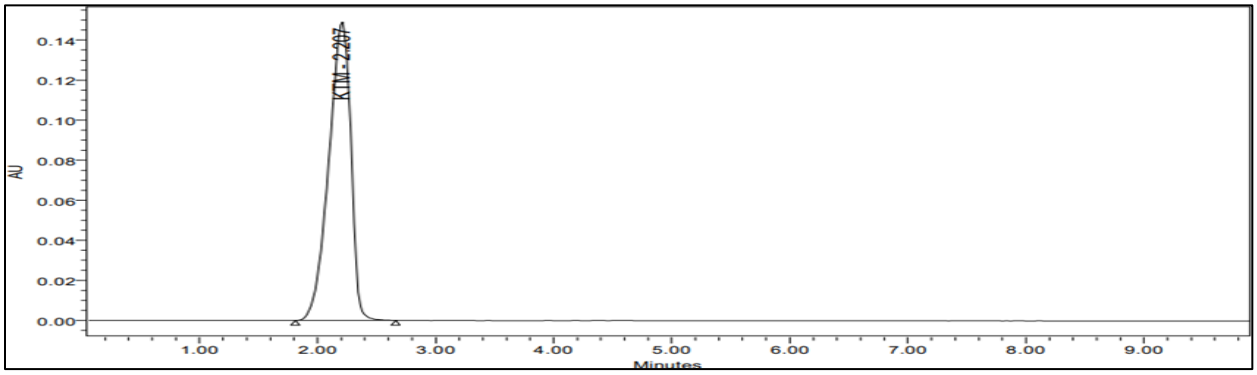

Figure 5: Standard chromatogram

Robustness Study:

Robustness studies were carried out using slight modification in chromatographic conditions like: Change in organic solvent (Methanol) concentration in mobile phase by ± 10 %. Change in flow rate by ± 0.1 ml/min. Change in pH by ±0.1 units. The above parameters were changed one by one and their effect was observed on test of assay study.

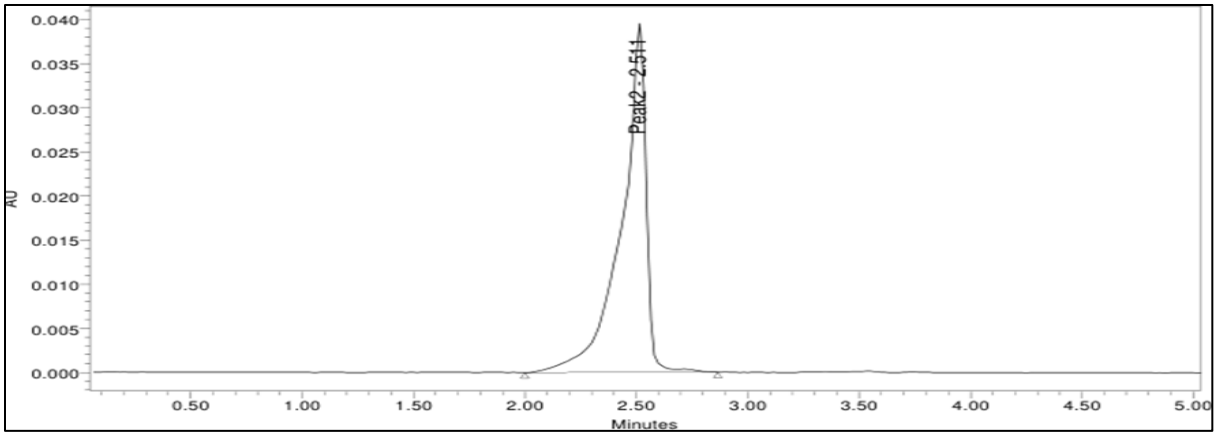

Figure 6: Robustness chromatogram for KT at flow rate 0.9 ml/min.

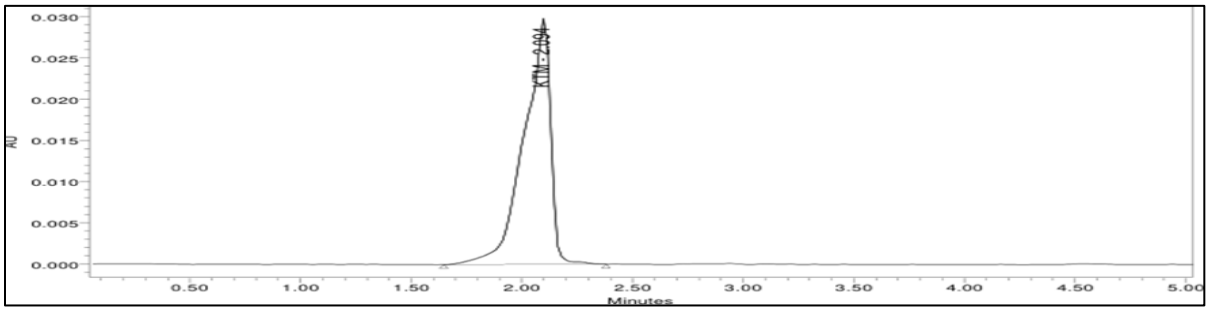

Figure 7: Robustness chromatogram for KT at flow rate 1.1 ml/min.

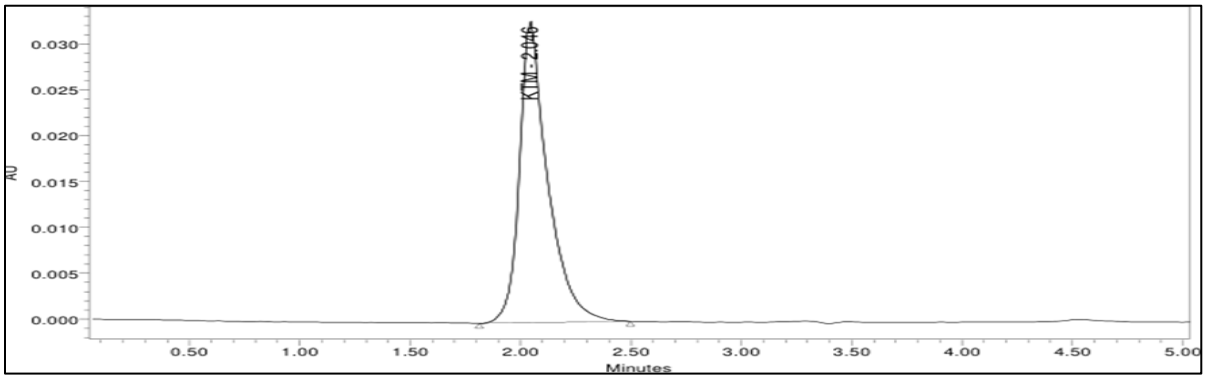

Figure 8: Robustness chromatogram for KT at mobile phase Methanol: ACN: Buffer (4:15:81)

Assay of prepared inhouse tablet of KT

Table 4: Assay study of KT tablet by standard addition method

Injection	Mean Area ± % RSD	Amount Recovered	% Recovered
1.	15140±1.08	1.950	98 %
2.	20048±2.0	3.902	98.5 %
3.	23154±1.05	4.995	99.4%
4.	25466±0.30	5.942	99.50 %
5.	26120±2.01	5.892	99 %

V. CONCLUSION

A robust, precise, accurate, and specific isocratic HPLC method was successfully developed and validated for the estimation of Ketorolac Tromethamine (KT) in matrix formulations. The method demonstrated excellent system suitability, with KT eluting at a retention time of 2.20 minutes. Validation parameters, including linearity (R^2 = 0.9994), accuracy (recovery of 98–99.5%), and precision, all met the acceptance criteria as per ICH guidelines. Therefore, the developed method is suitable for routine quality control and quantitative analysis of KT in pharmaceutical formulations.

VI. CONFLICTS OF INTERESTS

All authors have declared no conflict of interest.

REFERENCES

[1] ICH Harmonised Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology Q2(R1). International Conference on Harmonisation; 2005.

[2] Snyder LR, Kirkland JJ, Dolan JW. Introduction to Modern Liquid Chromatography. 3rd ed. Hoboken: John Wiley & Sons; 2010.

[3] Bakshi M, Singh S. Development of validated stability-indicating assay methods—critical review. J Pharm Biomed Anal. 2002;28(6):1011–1040.

[4] Puranik M, Wadher S, Bhangale BD, Jain DK. Development and validation of stability-indicating HPLC method for estimation of Ketorolac Tromethamine in pharmaceutical formulation. J Appl Pharm Sci. 2013;3(3):89–92.

[5] Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs—A review. J Pharm Anal. 2014;4(3):159–165.

[6] Sharma A, Arora S. Analytical method development and validation of Ketorolac Tromethamine in bulk and pharmaceutical dosage form by UV spectroscopy. Int J Pharm Sci Res. 2016;7(5):2130–2134.

[7] Swartz ME, Krull IS. Analytical Method Development and Validation. 1st ed. New York: CRC Press; 2012.

[8] Hemanthkumar M, Swamy G, Prasanthi D. RP-HPLC method development and validation for the simultaneous estimation of Ketorolac Tromethamine and Phenylephrine Hydrochloride. Int J Res Pharm Sci. 2020;11(1):1023–1027.

[9] Reddy BS, Swetha K, Venkatesh S. Development and validation of a RP-HPLC method for the estimation of Ketorolac Tromethamine in pharmaceutical dosage forms. Int J ChemTech Res. 2011;3(2):657–662.

[10] Patil VM, Patil VR. Development and validation of UV spectrophotometric method for estimation of Ketorolac Tromethamine in bulk and tablet formulation. Int J ChemTech Res. 2010;2(1):653–659.

[11] Rane VP, Sangshetti JN, Shinde DB. Stability-indicating RP-HPLC method for the determination of Ketorolac Tromethamine in bulk and pharmaceutical dosage form. Acta Pharm. 2010;60(4):507–516.

[12] Reddy PP, Suryanarayana MV, Reddy PN. Method development and validation for the simultaneous estimation of Ketorolac and Ofloxacin in ophthalmic dosage forms using RP-HPLC. J Pharm Res. 2011;4(7):2043–2045.

[13] Kumar S, Singh S, Singh R. RP-HPLC method development and validation for estimation of Ketorolac Tromethamine in ophthalmic dosage form. Int J Pharm Pharm Sci. 2012;4(Suppl 5):623–626.

[14] International Pharmaceutical Federation (FIP). Guidelines for Good Chromatographic Practice. FIP; 2014.

[15] FDA Guidance for Industry: Analytical Procedures and Methods Validation for Drugs and Biologics. U.S. Department of Health and Human Services, Food and Drug Administration; 2015.

[16] Rote AR, Barhate SD. Stability-indicating HPLC method for simultaneous determination of Ketorolac Tromethamine and Phenylephrine Hydrochloride in pharmaceutical formulations. J Appl Pharm Sci. 2012;2(6):168–172.

[17] Sethi PD. HPLC: Quantitative Analysis of Pharmaceutical Formulations. 1st ed. New Delhi: CBS Publishers; 2001.

[18] Singh G, Saini S. Analytical method development and validation of Ketorolac

Tromethamine in bulk and ophthalmic dosage form using HPLC. Der Pharmacia Lettre. 2016;8(14):69–75.

[19] Pandey S, Mishra A, Sahoo SK. Development and validation of a stability-indicating RP-HPLC method for the determination of Ketorolac Tromethamine. *Int J Pharm Pharm Sci.* 2013;5(4):604–609.

[20] Yadav D, Yadav S, Yadav S. Development and validation of RP-HPLC method for estimation of Ketorolac Tromethamine in bulk and pharmaceutical dosage form. *Int J Drug Dev Res.* 2011;3(2):271–275.

[21] Bhavsar AS, Patel PA, Patel MM. Stability indicating RP-HPLC method for simultaneous estimation of Ketorolac Tromethamine and Ofloxacin in ophthalmic dosage form. *J Pharm Sci Biosci Res.* 2011;1(1):34–38.