IJBPAS, December, 2026, 15(12): 6090-6109

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

SIMULTANEOUS QUANTIFICATION OF KETOROLAC TROMETHAMINE AND OFLOXACIN USING RP-HPLC BY QUALITY BY DESIGN

KHILARI SS[1*] AND BHANGALE CJ[2]

1: M. Pharm Department of Quality Assurance, PRES'S College of Pharmacy (for women), Chincholi, Nashik, Maharashtra, India

2: Ph.D., Department of Pharmaceutical Chemistry, PRES'S College of Pharmacy (for women), Chincholi, Nashik, Maharashtra, India

***Corresponding Author: Ms. Sakshi S. Khilari: E Mail: sakshikhilari99@gmail.com**

Received 29th May 2026; Revised 10th July 2026; Accepted 1st Oct. 2026; Available online 1st Dec. 2026

https://doi.org/10.31032/IJBPAS/2026/15.12.10700

ABSTRACT

A qualitative method for quantifying ketorolac tromethamine and ofloxacin in bulk and eye-drop formulations was developed and validated using quality-by-design principles, ensuring that it was simple, precise, reproducible, trustworthy, and accurate in classification. A response surface methodology with a two-level central composite design (CCD) was used. The study evaluated two factors: flow rate and mobile phase composition. Ketorolac tromethamine (16-80 µg/ml) and Ofloxacin (10-50 µg/ml) showed linearity across concentration ranges, with correlation values of 0.999 and 0.999, respectively. The limit of detection (LOD) and limit of quantification (LOQ) for ketorolac tromethamine were 2.0912 and 6.3371 µg/mL, respectively. Ofloxacin concentrations were 3.036 µg/mL and 0.22094 µg/mL for LOD and LOQ, respectively. The chromatographic separation was done using a methanol: water (0.1% OPA) mobile phase in the ratio of 72.2:27.8 V/V on an Agilent C18 column (250 mm × 4.6mm ID, 5 µm) under isocratic conditions, with a flow rate of 0.94 ml/min and UV detection at 312 nm. The RP-HPLC approach yielded a satisfactory retention time of 2.385 minutes for ketorolac tromethamine and 5.855 minutes for ofloxacin, which was optimized with Design-Expert version 13.0.1. The percentage recovery was determined to be 99-101%. The described procedures were found to be accurate and precise in assessing ketorolac tromethamine and ofloxacin in pharmaceutical preparations. These methods are suitable for ordinary analyses.

Keywords: RP-HPLC, QbD, Ketorolac Tromethamine and Ofloxacin, Development, Optimization, Validation

1. INTRODUCTION

Ketorolac tromethamine is known chemically as 1,3-dihydroxy-2-(hydroxymethyl)propan-2-aminium. 5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylate functions as an analgesic, an antipyretic, and a non-steroidal anti-inflammatory medication (NSAID). It is a non-selective inhibitor of cyclooxygenases (COX), inhibits both COX-1 and COX-2 enzymes, and prevents the inflammation process that turns arachidonic acid into prostaglandins **[1, 2]**. 10-oxo-4-oxa-1 7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl) Ofloxacin's chemical name is azatricyclo. [7.3.1.05,13] Fluoroquinolone trideca-5(13),6,8,11-tetraene-11-carboxylic acid is an antibiotic. Ofloxacin's main mode of action is inhibiting the bacterial DNA gyrase **[3–5]**. Several UV, HPLC, RP-HPLC, stability indicating HPLC, and HPTLC approaches have been published for both medicines alone and in various combinations, according to the literature review **[6–18]**. High-performance thin layer chromatography was used last for its analytical methods, per a study done on the combination of ofloxacin and ketorolac tromethamine **[19]**. Due to the increased sensitivity of high-performance liquid chromatography, we are currently concentrating on developing an HPLC method with an AQbD approach for validation. A methodical approach to development with a clear objective, Analytical Quality by Design (AQbD) emphasizes controlled method mastery based on solid science and quality risk management. Because the advanced risk assessment aids in identifying crucial analytical parameters that serve as the focus during the method development stage, the AQbD makes a substantial contribution to the creation of a strong technique. Because of its guiding principles, AQbD guarantees the methodology's quality across the course of the product's lifecycle **[20, 21]**. Several runs and a lot of time are needed for the typical optimization methods, which also need to be modified. In analytical techniques, QbD: Because of the growing emphasis on product quality and, by extension, patient safety, QbD in analytical methodology has gained more attention since its introduction in pharmaceutical product development, including analytical methods like RP-HPLC for quality control and analysis of active pharmaceutical ingredient (API) and drug product **[22, 23]**. In interlaboratory investigations, an AQbD technique may have two benefits: repeatability and method transfer. Furthermore, experimental design offered a superior substitute for the conventional method in the appropriate planning and execution of an optimization study **[24]**. In order to optimize the analytical method

concerning the design space where the response represents the optimum, several experimental designs have been carefully studied. Three-level factorial designs that are highly helpful include the Doehlert matrix design, Box–Behnken (BBD) design, and central composite design **[25, 26]**. Optimizing HPLC chromatographic conditions for CCD is the main goal of this effort. The CCD response surface design is determined by center replications of a hypercube and middle-pointing-edge sets of points. When developing an HPLC process, selecting the input parameters and the response or responses is a crucial stage. Through risk assessment and early testing, significant variables related to their effect on reaction were found. In order to identify and evaluate crucial variables that could pose a general threat to the procedure's performance, the Ishikawa (fishbone) diagram was employed in conjunction with preliminary experiments. CCD was used to improve certain factors because it was discovered that they significantly affected chromatographic separation, such as flow rate and methanol volume. With the use of the CCD experimental domain, these two effluent chromatographic factors—flow rate and methanol volume—were brought to actual values. Ofloxacin's retention time, resolution, tailing factor, and theoretical plates of ketorolac tromethamine were measured, as was their reaction to the measures. Therefore, the main objective of this work was to develop a simple, precise, comprehensive, and targeted approach for the quality control testing of the ofloxacin and ketorolac tromethamine combination utilizing CCD.

2. MATERIAL AND METHODS

2.1 Materials

Swapnroop Drug and Pharmaceutical in Maharashtra, India, provided gift samples of pure ofloxacin and ketorolac tromethamine. Merck Pvt. Ltd. (Mumbai, India) provided the HPLC-quality methanol (MeOH) and orthophosphoric acid, which are analytical reagent grade. A local pharmacy sold the KETOZOX-OF® eye drop formulation, which is said to include 0.3% OFLO and 0.5% w/v KETO. Preservatives like benzalkonium chloride solution might also be included.

2.2 Selection of wavelength by UV-visible spectrophotometer

Using a UV-Visible spectrophotometer, the isoabsorptive point of ketorolac, tromethamine, and ofloxacin was found to be 312 nm **(Figure 1)**, falling between 200 and 400 nm. Ketorolac 1600µg/ml and 1000µg/ml were the final concentrations obtained by diluting a standard stock solution containing 16 mg of ketorolac and 10 mg of ofloxacin with 10 ml of methanol. Before analysis, the stock solution was diluted with 10 ml of mobile phase to include KETO (16,32,48,64, and 80 µg/ml)

and OFLO (10,20,30,40, and 50 µg/ml). The UV-Visible Spectrophotometer was used to scan each solution in the spectrum mode between 400 and 200 nm in wavelength, and the spectra of each solution were superimposed. The wavelengths that were chosen were 297 nm OFX and 319 nm KRL.

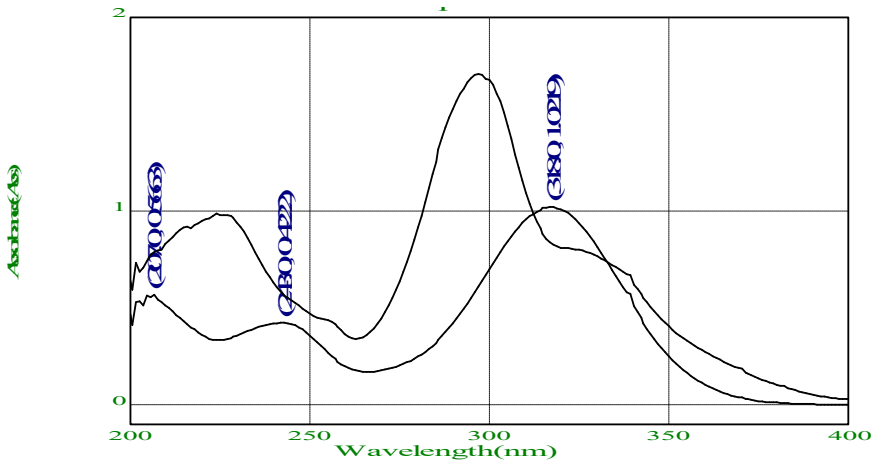


Figure 1: Iso-absorptive point of Ketorolac and Ofloxacin

2.3 Instrumentation

A UV (DAD) gradient detector and an auto-injector were features of an Agilent Technologies Gradient System used for a High-Performance Liquid Chromatography (HPLC) analysis. The system used ChemStation version 10.1 software and included a UV730D Absorbance detector, a 20 µl injection loop, and a Reverse Phase (Agilent) C18 column (4.6 mm x 250 mm; 5 µm).

2.4 Chromatographic Conditions

The Agilent reverse-phase C18 column (250 × 4.6 mm, 5 µm) was used for chromatographic separation. The mobile phase consisted of 0.1% orthophosphoric acid and 72.2:27.8 (v/v) methanol. Every day, the mobile phase was made, filtered using a nylon membrane filter with a pore size of 0.45 µm, and then sonicated for 15 minutes. The column was kept at room temperature while the mobile phase flow rate was tuned at 0.94 ml/min. The chromatogram was analyzed using a detection wavelength of 312 nm and an injection volume of 20 µl. The diluent was the mobile phase.

2.5 Preparation of Standard Solutions

Ketorolac tromethamine (1600 µg/ml) and ofloxacin (1000 µg/ml) were made independently in a standard stock solution. This required precisely weighing 10 mg of ofloxacin and 16 mg of ketorolac tromethamine, which were subsequently diluted in a 10 ml volumetric flask with some methanol. The mobile phase was used

to modify the final volume to the desired level. In a 10 ml volumetric flask, aliquots of ketorolac and ofloxacin were combined in a 1:2 ratio from this stock solution. The volume was also adjusted to the appropriate level using the mobile phase.

2.6 Preparation of Mobile Phase

Methanol and water (0.1% orthophosphoric acid) were combined in a volume ratio of 72.2:27.8 (v/v) to create the mobile phase.

2.7 AQbD-Based Method Development

In order to reduce method performance variability, the HPLC technique was developed utilizing AQbD principles to simultaneously quantify ofloxacin and ketorolac tromethamine in the recommended eye drop formulation. Method control strategy, working point selection and verification, experimental design, design space, critical parameter and response identification, risk assessment, and method objectives are all components of the AQbD methodology **(Figure 2)**.

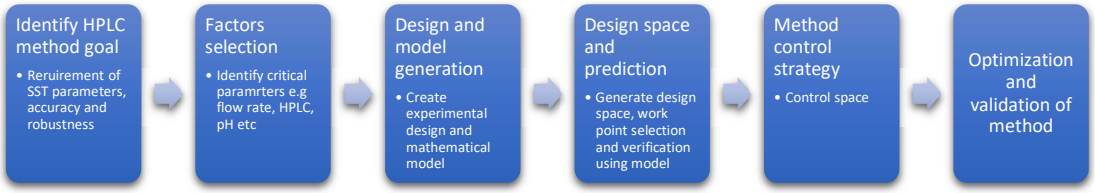


Figure 2: Graphical representation of method development flow using quality by design approach

2.8 Optimization of chromatographic conditions using CCD

Our understanding of how various chromatographic parameters impact separation quality is improved by the experimental design approach, which is helpful for separation optimization. **Figure 2** outlines a number of stages that must be taken in order to begin the process of creating an analytical method that adheres to Quality by Design (QbD) and to define its Design Space (DS) while optimizing the technique. In total, five steps are required to finish the method. Identifying the intended method's aim is the initial step, depending on the sort of technique being designed. For example, a conventional quality control assay process should prioritize speed and specificity. For this reason, we have set the same goals for our assay method. Two chromatographic variables and quantities selected to maximize the experimental condition are shown in **Table 1**. We coupled a partial factorial design with five replications of center points and five axial points at an extreme level to produce a Face-Centered Central Composite Design (FCCD) for two independent variables. The experimental results were fitted to the second-order model. Using the coefficient of determination R2, we evaluated the quality of the fitted polynomial models. We

were able to identify the genuine optimum state by using Derringer's desirability function, which simultaneously optimizes replies. Predicting the response and design space from the polynomial equation is the final step. When several independent variables, such as flow rate, pH, column temperature, etc., affect responses or dependent variables (e.g., peak tailing run time, resolution), a mathematical and statistical technique known as response surface methodology (RSM) might be helpful. In order to attain optimal system performance, this technique aids in simultaneously optimizing the levels of various variables. For any value of the chromatographic conditions in the experimental zone, RSM enables us to create quadratic models that explain the response. The optimization study employed a CCD for the second-order model, which requires the regression model coefficients to be computed at least three levels for every design variable.

Table 1: Experimental factors and levels used in the central composite design.

Factor	Name	Level(-)	Level (0)	Level(+)
A	Flow rate (ml/min)	0.8	1	1.2
B	Methanol (% v/v)	65	70	75

2.9 Validation of the method

The new method was validated for precision, accuracy, specificity, robustness, linearity, LOD, LOQ, and system applicability by the ICH Q2 (R1) requirements **[27]**.

i. **Test of system suitability**

Before sample analysis, the standard solution of ketorolac tromethamine and ofloxacin (100 mg/ml) was injected six times to determine the system's appropriateness. The system appropriateness results were based on resolution (R), theoretical plate (N), and tailing factor (T), which were calculated as a percentage RSD from six replicate drug injections.

ii. **Range and linearity**

Using five replicate measurements, the linear relationship between peak area and concentration of both medications was assessed over the concentration range of 10–60 µg/ml for ofloxacin and 16–96 µg/ml for ketorolac tromethamine. The analyte's highest and lowest concentrations were used to choose the analytical range where satisfactory linearity, accuracy, and precision were attained.

iii. **Limit of detection (LOD) and limit of quantitation (LOQ)**

Using the formulas limit of detection = 3.3 σ/S and limit of quantitation = 10 σ/S, where σ is the standard deviation of the response and S is the slope of a calibration curve, the LOD and LOQ of the developed method were determined from the standard

deviation of the response and the slope of the calibration curve of each drug by ICH guidelines.

iv. Precision

Repeatability and intermediate precision tests were used to examine the precision of the suggested approach. To determine intermediate precision, three replicates of three distinct concentrations 10, 30, and 50 µg/ml of ofloxacin and 16, 48, and 80 µg/ml of ketorolac tromethamine were examined on separate days and on the same day for repeatability. The overall mean and percentage RSD were computed.

v. Accuracy

The methodological recovery studies evaluated the accuracy of the optimized approach in determining the recovery of both medications at varying levels. For each drug, a known quantity of standard was added to the designed formulation at 80, 100, and 120% levels. The results were then examined twice using the suggested approach. The created formulation (100 µg/ml) was spiked with three different doses of ketorolac tromethamine standard (80, 100, and 150 µg/ml) using the standard addition method in order to conduct recovery experiments. Similar to this, recovery experiments for ofloxacin were conducted by using the standard addition method to spike three distinct amounts of simvastatin standard (80, 100, and 120 µg/ml) to the synthetic mixture (100 µg/ml). % Recovery was then calculated for both drugs.

vi. Robustness

A number of elements were purposefully altered, such as the volume of methanol (71.2 and 73.20 ml) and the wavelengths (311 nm and 313 nm), in order to test the ability of this recently designed analytical approach to tolerate minor, intentional alterations in the method.

vii. Specificity

To identify excipient interference, the chromatogram of the synthetic combination that represented the formulation was compared to the blank and standard solution of the same medications. Both ofloxacin and ketorolac tromethamine must have no discernible interference in the excipient chromatogram during the retention period.

b. **Analysis of Marketed Formulation**

Ketorolac tromethamine and ofloxacin were measured in the developed eye dosage form using the developed HPLC method. The developed formulation, which contained 0.3% w/v ofloxacin and 0.5% w/v ketorolac tromethamine, was analyzed. 5 ml of 0.5% ketorolac and 0.3% ofloxacin should be dissolved in 10 ml of methanol. Add roughly 10 milliliters of methanol to the diluents, sonicate to dissolve them fully, and then add enough diluent to reach the desired amount. After thoroughly mixing, strain over a 0.45 µm filter. Additionally, pipette 0.3 ml of the stock solution mentioned above into a 10-ml

volumetric flask and use diluents to dilute it to the appropriate level (48 + 30 µg/ml). For ketorolac tromethamine and ofloxacin, the final concentrations were µg/ml, respectively. For ofloxacin and ketorolac tromethamine, the final concentrations were 30 and 48 µg/ml, respectively. Fresh working sample solutions were made by appropriately diluting the stock solution with the mobile phase to achieve final concentrations of 20 µg/ml for ofloxacin and 40 µg/ml for ketorolac tromethamine. After injecting this solution into the HPLC system, the previously mentioned method was used to determine the peak area, and the same process was followed twice for analysis.

c. Statistical analysis

Microsoft Excel 2007 was used for the statistical study, which included calculating the mean, relative standard deviation, standard deviation, and linear regression analysis. Response surface optimization with ANOVA and the creation of a 3D response surface plot and perturbation plot were done using Design Expert software version 13.0.1. To assess the homoscedasticity of variance and deviation from linearity, a lack-of-fit test was applied to the linearity data **[28]**.

3. RESULTS

3.1 AQbd assisted with method development.

Analytical quality by design (AQbD) aimed to satisfy requirements by separating both peaks in a shorter amount of run time while maintaining a satisfactory peak form. The HPLC method's accuracy and precision, with a % RSD less than, were secondary objectives, leading to a robust approach with minimal variability. In order to separate the two medications in a short amount of time, the mobile phase conditions were optimized. In order to satisfy the system suitability parameter, a number of mobile phases and their various ratios, including acetonitrile, methanol, potassium dihydrogen orthophosphate buffer, di-sodium hydrogen orthophosphate buffer, and their combinations with varying pH, were tested based on a review of the literature. After experimenting with different ratios of di-sodium hydrogen orthophosphate buffer pH 2.9: acetonitrile, the ratio of methanol and water (0.1% OPA)72.2:27.8 was superior in every way, with the exception of solvent interference. Different pH values of potassium dihydrogen orthophosphate buffer were explored; however, when the buffer's pH rose, it was found that the ofloxacin peak widened. After that, the sample was made using the mobile phase as a diluent to eliminate solvent interference. Therefore, based on acceptable system compatibility characteristics, preliminary studies showed that the mobile phase composition, methanol: orthophosphoric

acid, was appropriate. Furthermore, in early tests, the volume of methanol significantly altered the retention time. Critical parameters chosen for further investigation were the volume of methanol (ml) as method variables and the flow rate (ml/min) as an instrumental parameter, which were found to have the most significant impact on system suitability parameters based on the Ishikawa diagram and preliminary experiments **(Figure 3)**. A set of points located at the middle of each edge of a multidimensional cube and center replicates define the CCD response surface design for optimization.

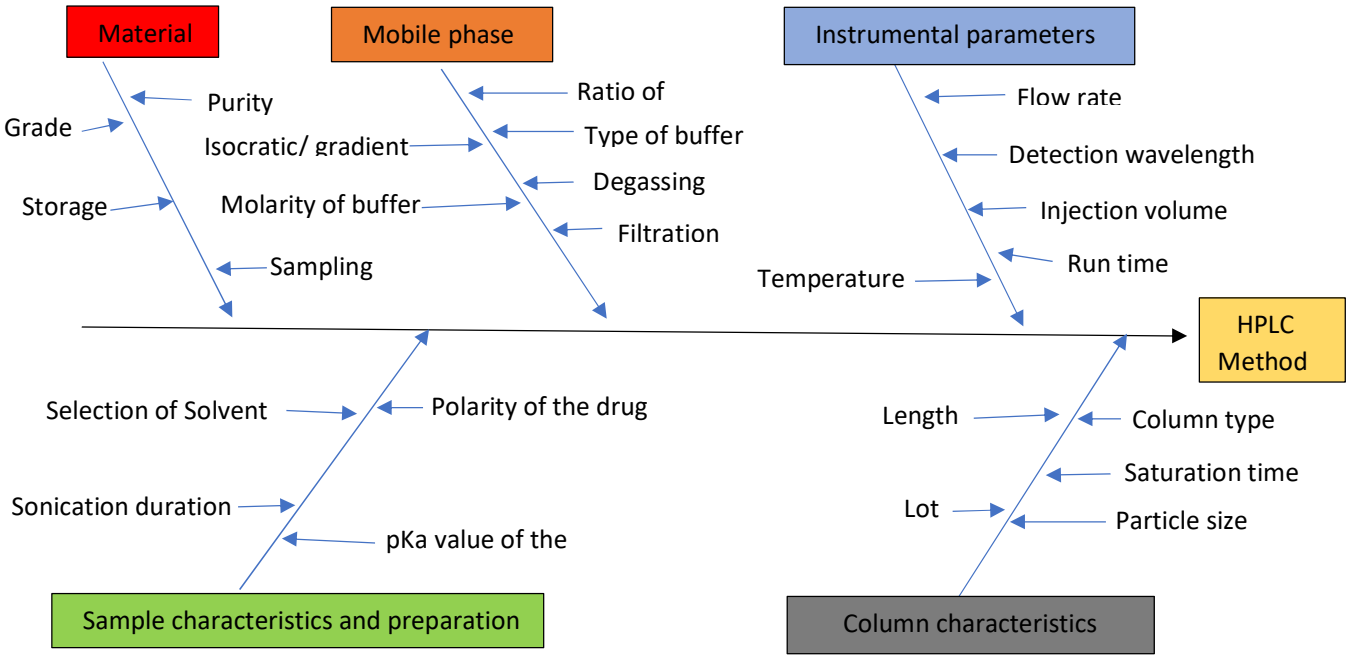


Figure 3: The Ishikawa (fishbone) diagram to identify potential variables in HPLC method development

CCD was used to optimize chromatographic conditions by considering the effects of different factors such as volume of methanol (A), and flow rate (B) on retention time of ketorolac tromethamine (R1), area of ketorolac tromethamine (R2), theoretical plates of ketorolac tromethamine (R3), retention time of ofloxacin (R4), area of ofloxacin (R5), theoretical plates of ofloxacin (R6). **Table 2** represents the effect of all these two variables on responses like on retention time of ketorolac tromethamine (R1), area of ketorolac tromethamine (R2), theoretical plates of ketorolac tromethamine (R3), retention time of ofloxacin (R4), area of ofloxacin (R5), were selected as responses/CQAs. The model was also validated by analysis of variance (ANOVA) using Design Expert software, and the results are presented in **Table 3**. Based on PRESS value, the quadratic model was selected for on retention time of ketorolac

tromethamine (R1), area of ketorolac tromethamine (R2), theoretical plates of ketorolac tromethamine (R3), retention time of ofloxacin (R4), and area of ofloxacin (R5). Significant effects showed a p-value less than 0.05. Adequate Precision, defined as a signal-to-noise ratio greater than 4, is desirable, and the obtained ratio for all the responses indicated an adequate signal **Table 3**. The low standard deviation (% CV) and high adjusted R-squared value indicated a good relationship between the experimental data and those of the fitted models. The predicted R-squared value was in acceptable concordance with the adjusted R-squared value for all responses. The final equation, in terms of actual components and factors, which can be used to make predictions about the response for given levels of each factor, are for retention time of Ketorolac tromethamine **(R1)**, +2.38-0.0243*A 0.1830*B+0.0325*A*B+0.0912*A^2+0.0055*B^2; for area of ketorolac tromethamine **(R2)**, +430.51+8.22*A-32.45*B+1.87*A*B+13.88*A^2+2.51*B^2; for theoretical plates of ketorolac tromethamine **(R3)**,+5229.80+911.80*A-157.34*B-200.25*A*B-548.65*A^2-24.40*B^2; for retention time of ofloxacin **(R4)**, +5.81-1.52*A-0.4684*B+0.0808*A*B*+0.9315*A^2-0.0395*B^2; for area of ofloxacin **(R5)**, +412.77+13.88*A-42.60*B-7.07*A*B+15.68*A^2-4.21*B^2. A positive value represents an effect that favours the optimization, while a negative value indicates an inverse relationship between the factor and the response.

A response surface was constructed to evaluate the effect of the factors on selected responses. Change in flow rate resulted in a decrease in the resolution of ofloxacin at both lower level and higher level from the reference point. Change in volume of methanol resulted in an increase in the resolution of ofloxacin at a lower level and a decrease in the resolution of ofloxacin at a higher level from the reference point. As shown in **Figure 4. (a-e)**, the three-dimensional graphs of RSM are generated as a function of the significant variables, usually the proposed optimum. The representative plots for responses are presented in Figure 4 (a-e), in which the interaction between the variables (factors A and B) and their mutual dependence is observed. **Figure 4 (a) and (b)** indicate that flow rate showed a prominent effect on the retention time of ketorolac tromethamine and ofloxacin. The retention time of ketorolac tromethamine increased with a decrease in flow rate. The resolution of ofloxacin increased with an increase in flow rate up to a certain value. **Figure 4 (c)** demonstrates that the theoretical plates of ketorolac tromethamine increase with an increase in flow rate. **Figure 4 (d)** represents that the retention time of ofloxacin increased

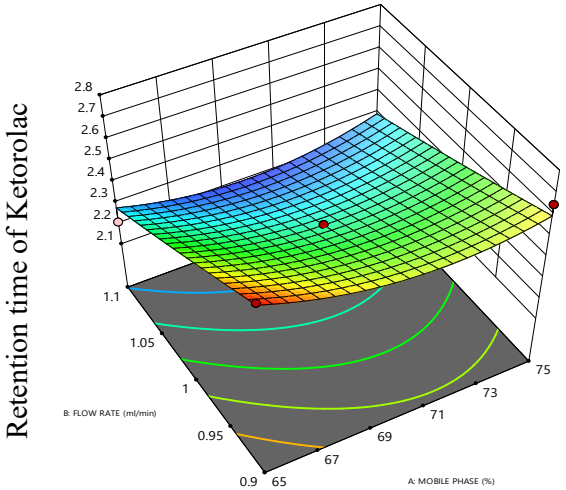
with a decrease in flow rate. **Figure 4 (e)** depicts that the flow rate showed no prominent effect on the peak area of ofloxacin, where the peak area of ofloxacin increased with a decrease in flow rate.

Table 2: Experimental design domain (CCD) for each run with responses

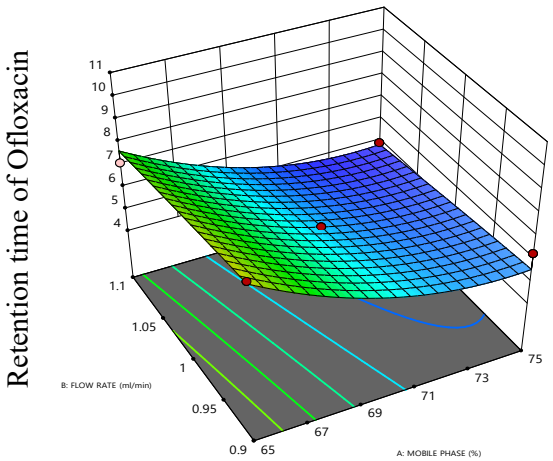
Run	Factors		Responses							
	Flow rate (A) (ml/min)	Mobile phase (B) (ml)	Rt of KETO (R1)	Resolution of KETO (R2)	Theoretical plates of KETO (R3)	Tailing factor of KETO (R4)	Rt of OFLO (R5)	Resolution of OFLO (R6)	Theoretical plates of OFLO (R7)	Tailing factor of OFLO (R8)
1	70	1	2.376	431.552	5228	0.53	5.793	410.747	8143	0.62
2	70	1	2.379	429.257	5244	0.54	5.814	411.906	8448	0.62
3	70	1.1414	2.195	404.038	4789	0.54	5.426	337.780	7582	0.65
4	70	0.8586	2.601	475.869	5486	0.55	5.862	455.978	8954	0.67
5	77.0711	1	2.505	467.741	5433	0.53	5.00	448.822	8351	0.59
6	65	1.1	2.210	388.233	3959	0.47	7.136	376.216	7712	0.67
7	70	1	2.377	433.268	5230	0.53	5.809	408.124	8438	0.62
8	70	1	2.374	428.358	5225	0.54	5.812	413.598	8456	0.63
9	62.929	1	2.634	457.654	2745	0.42	10.17	424.501	8329	0.47
10	65	0.9	2.720	471.006	3695	0.43	8.863	448.892	8639	0.66
11	75	1.1	2.269	417.725	5305	0.55	4.873	400.419	8005	0.61
12	70	1	2.375	430.128	5222	0.54	5.818	419.475	8462	0.63
13	75	0.9	2.649	493.006	5842	0.51	6.277	501.358	8891	0.59

Table 3: ANOVA regression analysis for models and responses

Response	Standard deviation	Mean	% C.V.	PRESS	r^2	Adjusted R-square	Predicted R-square	Adequate precision	p value
Retention time of keto	0.0494	2.44	2.03	0.0171	0.9516	0.9170	0.6557	17.4247	0.0056
Peak area of keto	10.30	440.60	2.34	742.92	0.9329	0.8849	0.5302	13.7268	0.0269
Theoretical plates of keto	113.90	4877.15	2.34	90809.97	0.9901	0.9831	0.9301	39.8445	0.0001
Retention time of oflox	0.4795	3.36	7.54	1.61	0.9427	0.9018	0.5928	15.4376	0.0039
Peak area of oflox	10.34	419.83	2.46	747.73	0.9606	0.9325	0.7411	20.4626	0.0168
Theoretical plates of oflox	117.23	8339.23	1.41	76335.33	0.9292	0.9150	0.8909	23.5638	0.7646



(a)



(b)

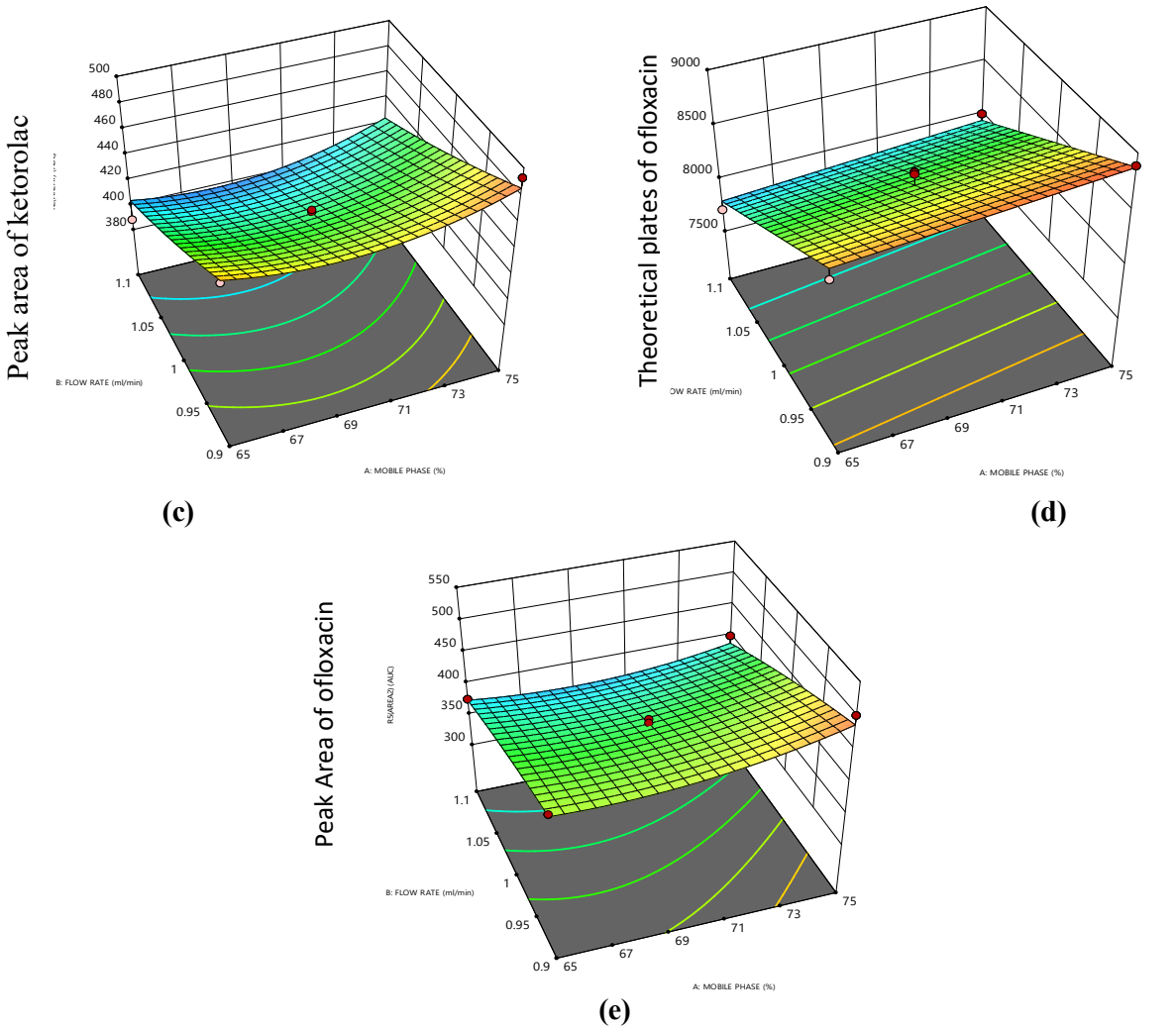


Figure 4 (a-e) 3-Dimensional plot of RSM showing effect of factors (a) Retention time of ketorolac, (b) Retention time of Ofloxacin, (c) Peak area of ketorolac, (d) Theoretical plates of ofloxacin, (e) Area of ofloxacin

During numerical optimization, the design offered 13 solutions for optimized chromatographic conditions, but the solutions were reduced by setting the goals. Various constraints for factors and responses were set, and three solutions were selected for optimization of chromatographic conditions. It was demonstrated that the observed value of optimized chromatographic conditions was quite close to the predicted value, resulting in less % error and high desirability. Hence, the optimized condition; flowrate (0.94ml/ min) and volume of methanol (78.2 ml) resulted in retention time of ketorolac tromethamine and ofloxacin at 2.385 and 5.855min, respectively **(Figure 5)**. Design space issued for establishment of a multidimensional space based on method factors and settings; design space hence can provide suitable method performance in terms of system suitability and retention time.

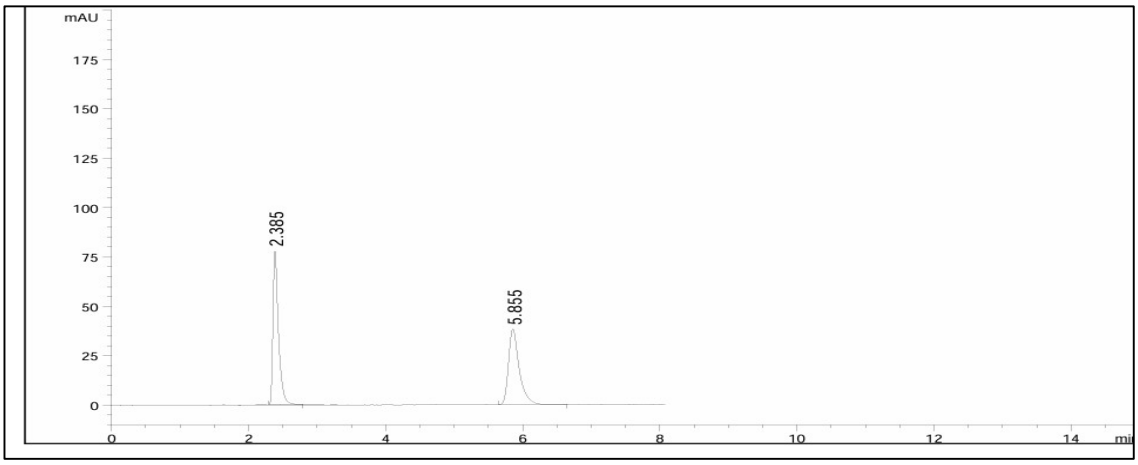


Figure 5: Optimized HPLC chromatogram for Ketorolac Tromethamine and Ofloxacin at 312 nm

3.2 Validation of the method

System suitability testing is used as a method of control strategy. System suitability tests are an integral part of method development and were performed to evaluate the behaviour of the chromatographic system. The results of system suitability testing, retention time (2.385±0.635 for KETO and 5.855±0.686 for OFLO), resolution(12.3±0.877), theoretical plate (5269±0.147 for KETO and 8078±0.499 for OFLO), and % RSD evaluated for six replicate injections were found to be acceptable. Ketorolac tromethamine and Ofloxacin showed a good correlation coefficient (r2=0.999 for Ketorolac tromethamine and 0.999 for Ofloxacin, **Figure 6, 7**) in the given concentration range 16-80 µg/ml for Ketorolac tromethamine and 10–50 µg/ml for Ofloxacin, respectively **(Table 4, 5)**. The LOD and LOQ were found to be 2.0912 and 6.3317 mg/ml, respectively, for ketorolac tromethamine and 3.036 and 0.22094 µg/ml, respectively, for ofloxacin. Repeatability and intermediate precision were carried out by performing two replicates of concentration (48 µg/ml of ketorolac tromethamine and 30 µg/ml of ofloxacin) **(Table 6)**, which showed % RSD less than 2%, indicating acceptable precision in terms of repeatability of peak area measurement and sample application. The percentage recovery for both the drugs at three concentration levels (80, 100, 120 %) was found in the range of 99 –101 %, suggesting the suitability of the method to perform routine drug analysis **(Figure 8)**. The robustness **(Tables 7, 8)** of the proposed method was evaluated by making small deliberate variations in liquid chromatography conditions. Moreover, the method was found to be specific as the excipients did not produce any interference at the retention time of both drugs. In this study, the chromatographic parameters monitored were retention time and area. The results of the analysis of robustness, where

% RSD is less than 2, indicated that the method is robust and is not affected by small changes in routine use.

Table 4: Linearity of Ketorolac Tromethamine

Concentration µg/ml	Area Ketorolac tromethamine
16	675.186
32	851.552
48	1055.910
64	1261.979
80	1457.819

Table 5: Linearity of Ofloxacin

Concentration µg/ml	Area Ofloxacin
10	389.323
20	840.696
30	1213.786
40	1669.509
50	2056.949

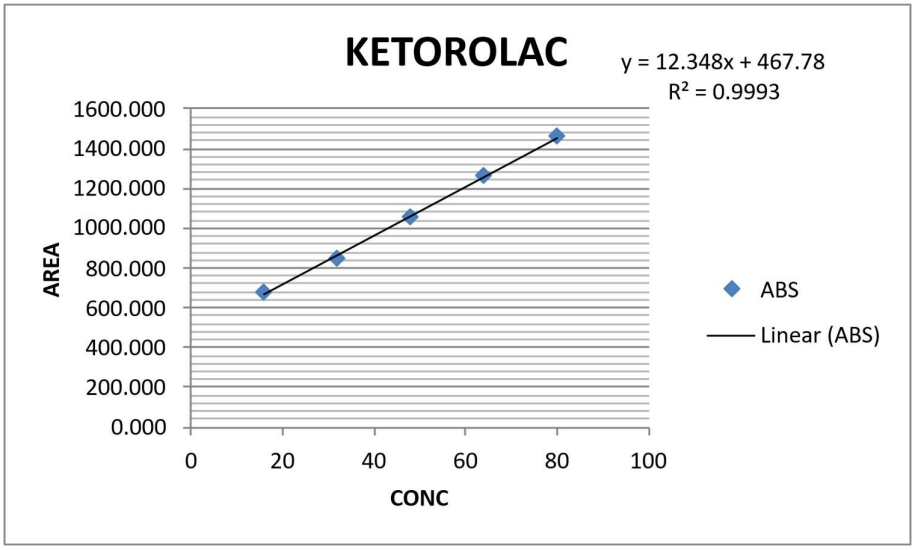


Figure 6: Calibration curve of Ketorolac Tromethamine

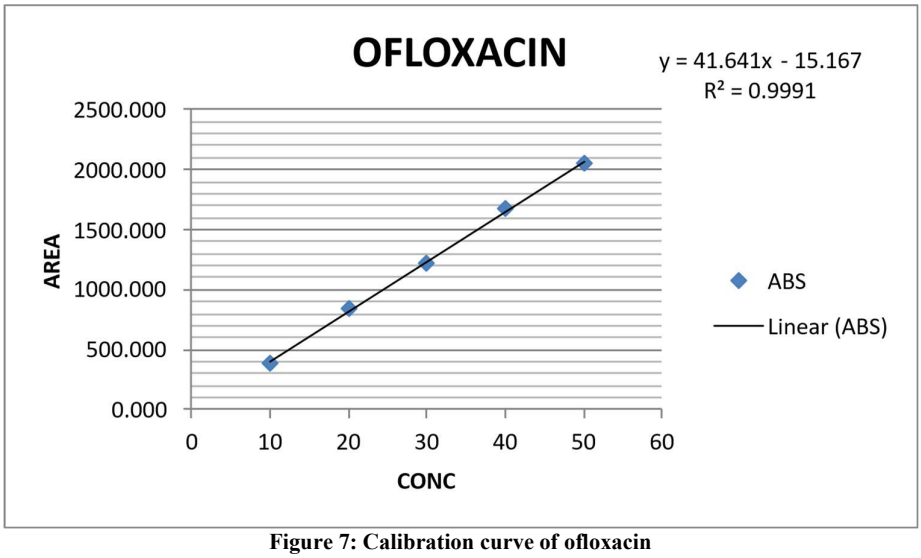


Figure 7: Calibration curve of ofloxacin

Table 6: Result of Intraday and Interday Precision studies on RP-HPLC for Ketorolac and Ofloxacin

Drug	Conc^n (µg/ml)	Intraday Precision		% RSD	Interday Precision		% RSD
		Mean± SD	%Amt Found		Mean± SD	%Amt Found	
KRL	16	667.12± 2.90	101.00	0.43	668.07± 1.49	101.48	0.22
	48	1057.9±1.91	99.64	0.18	1054.63±3.66	99.09	0.35
	80	1088.1±3.61	99.28	0.09	1448.25±1.30	99.33	0.09
OFX	10	397.54±1.22	99.11	0.31	398.18±0.45	99.27	0.36
	30	1228.4±1.42	99.55	0.12	1227.85±2.60	99.50	0.21
	50	2056.8±0.52	99.52	0.03	2056.39±2.91	99.50	0.14

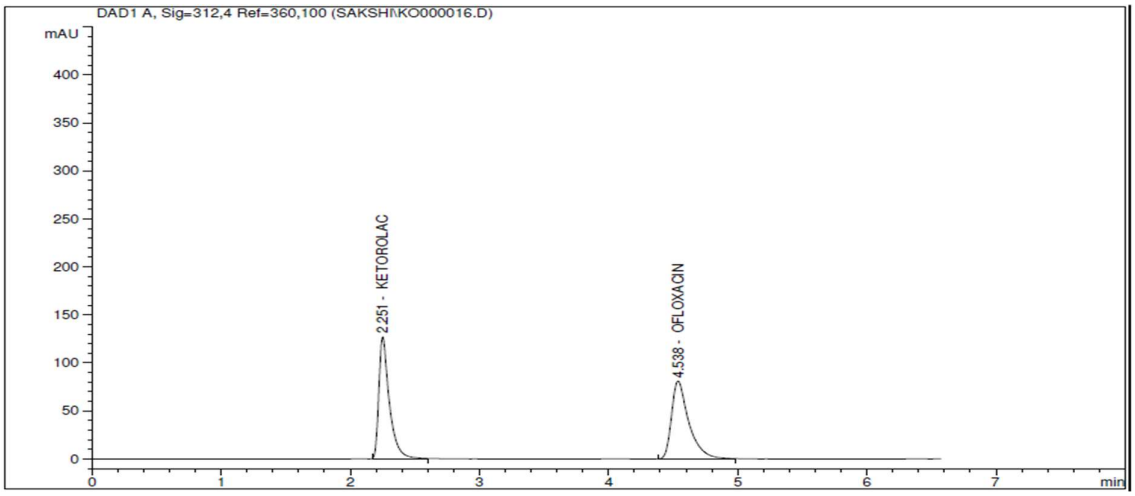
***The mean of each of the 3 concentrations has 2 readings.**

Table 7: Result of Robustness Study of Ketorolac Tromethamine

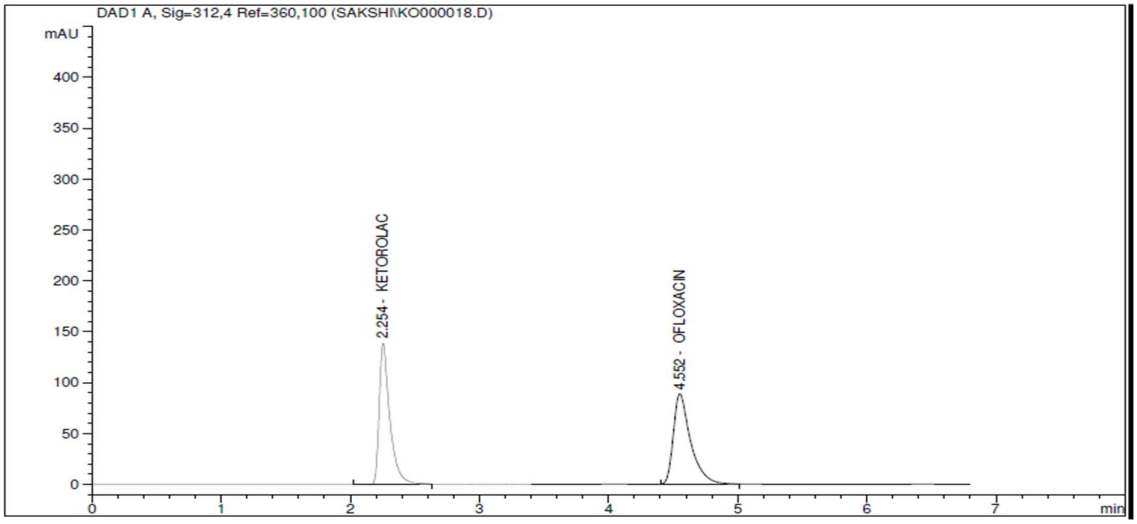
Parameters	Conc.(µg/ml)	Amount of detected (mean ±SD)	%RSD
Chromatogram of comp change 71.2 MEOH +28.8 WATER	32	806.08±1.47	0.18
Chromatogram of comp change 73.2 MEOH + 26.8 WATER	32	806.90±0.99	0.12
Chromatogram of comp change wavelength change 311 nm	32	856.2±0.15	0.02
Chromatogram of comp change wavelength change 313 nm	32	765.69±1.23	0.16

Table 8: Result of Robustness Study of Ofloxacin

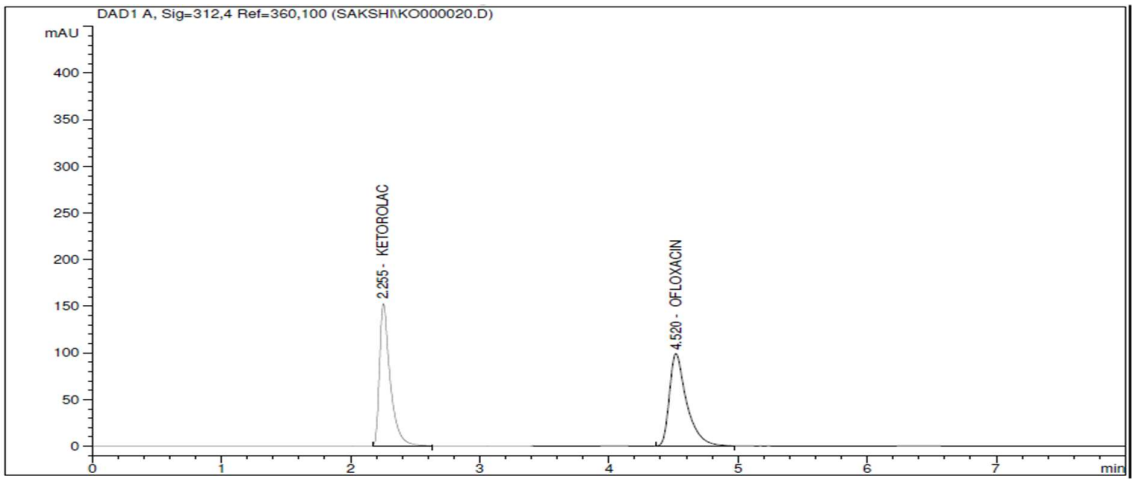
Parameters	Conc.(µg/ml)	Amount of detected (mean ±SD)	%RSD
Chromatogram of comp change 71.2 MEOH +28.8 WATER	20	840.59±0.64	0.08
Chromatogram of comp change 73.2 MEOH + 26.8 WATER	20	842.41±0.16	0.02
Chromatogram of comp change wavelength change 311 nm	20	830.3±0.001	0.01
Chromatogram of comp change wavelength change 313 nm	20	847.58±0.39	0.05



(a)



(b)



(c)

Figure 8: Chromatogram of Accuracy (a) 80% (b) 100% (c) 120%

3.3 Analysis of developed formulation

The developed HPLC method in the present study was used to quantify ketorolac tromethamine and ofloxacin in the developed eye drop dosage form. The percentage amount of drug found on twice analysis for both ketorolac tromethamine and ofloxacin was found to be 99.74% and 99.08 % respectively, and hence the developed method can be routinely used for analysis of ketorolac tromethamine and ofloxacin **(Table 9)**.

Table 9: Analysis of marketed formulation

Assay	Drug	Label Claimed	Amt. Found	% Label Claim	SD	%RSD
Rp-HPLC	KRL	48	1057.055	99.50	0.030	0.062
	OFX	30	1243.87	98.36	0.015	0.051
	KRL	48	1057.573	99.59	0.062	0.062
	OFX	30	1242.991	98.29	0.050	0.051

4. DISCUSSION

A simple, sensitive, accurate, robust, economical, and precise HPLC analytical method using a QbD approach has been developed for the simultaneous determination of ketorolac tromethamine and ofloxacin in a developed eye drop dosage form. Using the QbD approach, the experimental design allowed evaluating the selected factors simultaneously, including interactions between factors, by means of a rational approach in order to reach the optimum conditions. Flow rate was found to be the most significant factor for the retention time of ketorolac tromethamine and ofloxacin, as well as for the resolution of ofloxacin by CCD. Flow rate and volume of methanol both were found to have a profound effect on the theoretical plates of ketorolac tromethamine, while volume of methanol was the most important factor for the tailing factor of ofloxacin. It is concluded that the use of experimental design and a decision-making approach is a flexible procedure, able to reduce the number of needed experiments for the development and optimization of an HPLC method, which can generate maximum information in less time with a small number of experiments. The good % recovery in formulation suggests that the excipients present in the formulation have no interference in the determination. The validation study supported the selection of the best conditions by confirming that the method was specific, accurate, linear, precise, and robust. Therefore, the same methodology can be applied for quality control testing and estimation of ketorolac tromethamine and ofloxacin.

ACKNOWLEDGMENTS

The authors are grateful to PRES's College of Pharmacy, Chincholi. Nashik, for providing the necessary facilities, instrumentation, and academic support to carry out this research. The authors also acknowledge the faculty and staff for their valuable assistance and encouragement throughout the study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not for profit sectors.

REFERENCES

[1] Shukla, T.; Pandey, S.P.; Khare, P.; Upmanyu, N., Development of ketorolac tromethamine loaded microemulsion for topical delivery using D-optimal experimental approach: Characterization and evaluation of analgesic and anti-inflammatory efficacy., Journal of Drug Delivery Science and Technology, 64 (1), 2021, 102632.

[2] Buckley, M. M.; Brogden, R.N., Ketorolac: a review of its pharmacodynamic and pharmacokinetic

properties, and therapeutic potential., Drugs., 39(1), 1990, 86-109.

[3] Sahoo, M.S.; Ujwal, M.U.; Bhatra, M.K.; Rani, M.S., Ofloxacin: a literature review on analytical and bio-analytical methods., The International Journal of Analytical and Experimental Modal Analysis., 14(12), 2022, 548-557.

[4] Drew, R.H.; Gallis, H.A., Ofloxacin: its pharmacology, pharmacokinetics, and potential for clinical application. Pharmacotherapy, The Journal of Human Pharmacology and Drug Therapy., 8(1), 1988, 35-46.

[5] Sumithra, M., Determination of chromatographic assay and validation of ofloxacin in bulk and pharmaceutical dosage form., Asian Journal of Pharmaceutical and Clinical Research., 11(4), 2018, 98-101.

[6] Vichare, V.; Tambe, V.; Joshi, S.V.; Dhole, S.N., New Simultaneous UV-Visible Spectrophotometric Methods for Estimation of Ofloxacin and Ketorolac Tromethamine in Ophthalmic Dosage Form., Asian Journal of Pharmaceutical Analysis., 3(2), 2013, 53-57.

[7] Mansoori, M.H.; Sahu, B.; Shah, K., Simultaneous Estimation of Ketorolac Tromethamine and Ofloxacin., Research Journal of Pharmacy and Technology.,15(6), 2022, 2498-2502.

[8] Fegade, J.D.; Mehta, H.P.; Chaudhari, R.Y.; Patil, V.R., Simultaneous spectrophotometric estimation of ofloxacin and ketorolac tromethamine in ophthalmic dosage form., International Journal of Chem. Tech Research., 1(2), 2009, 189-194.

[9] Patila, S.B.; Moreb, J.D., Revolutionizing Ophthalmic Drug Analysis: Simultaneous Quantification of Ofloxacin and Ketorolac Tromethamine using Reverse Phase High Performance Liquid Chromatography., Natural Volatiles & Essential Oils., 8(6), 2021, 6637-6643.

[10] Ramakrishna, V.; Satyanarayana, P.; Haribabu, B., Development and validation of a liquid chromatographic method for the simultaneous estimation of ofloxacin and ketorolac tromethamine in combined dosage form., American Journal PharmTech Research., 4(2), 2014, 282-291.

[11] Razzaq, S.N.; Ashfaq, M.; Khan, I.U.; Mariam, I., Stability indicating HPLC method for the simultaneous determination of ofloxacin and ketorolac tromethamine in pharmaceutical formulations., Analytical methods., 4(7), 2012, 2121-2126.

[12] Demircan, S.; Sayın, F.; Başcı, N.E.; Unlu, N.; Kır, S., Determination of ketorolac tromethamine in human eye samples by HPLC with photo diode-

array detection., Chromatographia., 66(1), 2007, 135-139.

[13] Gade, B.R.; Bandhakavi, S.R.; Ramanaiah, G.; Satyanarayana, M.V., Method development and validation of stability indicating RP-HPLC method for simultaneous estimation of ofloxacin and ketorolac tromethamine in bulk and its pharmaceutical formulations, American Journal of Advanced Drug Delivery., 2(6), 2014, 786-801.

[14] Pawar, S.; Punekar, A.; Gosavi, S.; Gorde, P.; Harak, S., Development and validation of RP-HPLC method for determination of ketorolac tromethamine from bulk and pharmaceutical formulations., International Journal of Health Sciences., 6(s2), 2022, 14440– 14447.

[15] Hussain, N.; Shah, N.H.; SYED, S.K.; Rasool, M.F.; Nisar, N.; Majeed, A., A Simple and Sensitive HPLC-UV Method for the Estimation of Ofloxacin in Plasma., Latin American Journal of Pharmacy., 40(11), 2021, 2731-2734.

[16] Sireesha, K.R.; Prakash, K., HPLC-UV method for simultaneous determination of ofloxacin and dexamethasone sodium phosphate., International Journal of Pharmacy and Pharmaceutical Sciences., 4(1), 2012, 415-418.

[17] Sunil, G.; Jambulingam, M.; Thangadurai, S.A.; Kamalakannan, D.; Sundaraganapathy, R.; Jothimanivannan, C., Development and validation of ketorolac tromethamine in eye drop formulation by RP-HPLC method., Arabian Journal of Chemistry., 10(1), 2017, S928-S935.

[18] Razzaq, S.N.; Khan, I.U.; Ashfaq, M.; Mariam, I., Stability indicating HPLC method for simultaneous determination of moxifloxacin hydrochloride and ketorolac tromethamine in pharmaceutical formulations., Química Nova, 35(6), 2012, 1216-1221.

[19] Gandhi, S.P.; Dewani, M.G.; Borole, T.C.; Damle, M.C., Development and validation of an indicating HPTLC method for the determination of Ofloxacin and Ketorolac Tromethamine in combination., Journal of Advanced Scientific Research., 2(3), 2021, 77-82.

[20] Bhatt, D.A.; Rane, S.I., QbD approach to analytical RP-HPLC method development and its validation., International Journal of Pharmacy and Pharmaceutical Sciences, 3(1), 2011, 179-187.

[21] Bhutani, H.; Kurmi, M.; Singh, S.; Beg, S.; Singh, B., Quality by design (QbD) in analytical sciences: an overview., Quality Assurance, 46(8), 2004, 46(08), 71-75.

[22] Awotwe-Otoo, D.; Agarabi, C.; Faustino, P.J.; Habib, M.J.; Lee, S.; Khan, M.A.; Shah, R.B., Application of quality by design elements for the development and optimization of an analytical method for protamine sulfate., Journal of Pharmaceutical and Biomedical Analysis, 62, 2012, 61-67.

[23] Patel, K.G.; Patel, A.T.; Shah, P.A.; Gandhi, T.R.; Multivariate optimization for simultaneous determination of aspirin and simvastatin by reverse phase liquid chromatographic method using AQbD approach, Bulletin of Faculty of Pharmacy, Cairo University, 55(2), 2017, 293-301.

[24] Schmidt, A.H.; Stanic, M.; Molnár, I., In silico robustness testing of a compendial HPLC purity method by using a multidimensional design space built by chromatography modeling—Case study pramipexole., Journal of Pharmaceutical and Biomedical Analysis, 91(1), 2014, 97-107.

[25] Patel, K.G.; Shah, P.M.; Shah, P.A.; Gandhi, T.R., Validated high-performance thin-layer chromatographic (HPTLC) method for simultaneous determination of nadifloxacin, mometasone furoate, and miconazole nitrate cream using fractional factorial design., Journal of food and drug analysis, 24(3), 2016, 610-619.

[26] Solanki, T.B.; Shah, P.A.; Patel, K.G., Central composite design for validation of HPTLC method for simultaneous estimation of olmesartan medoxomil, amlodipine besylate, and hydrochlorothiazide in tablets., Indian Journal of Pharmaceutical Sciences., 76(3), 2014, 179-187.

[27] ICH harmonized tripartite guideline., Validation of analytical procedures: text and methodology Q2 (R1), in: International Conference on Harmonization, Geneva, Switzerland, 2005.

[28] Zar J.H., Biostatistical analysis, Pearson Education India, 4th ed, 1999, 1-482.