Section A-Research Paper

Application of Qbd Approach for specific Analytical method development for estimation of COX 2 inhibitor- Ketorolac in tablet dosage form

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Abstract

The aim of the present work was to change from the quality by testing (Qbt) prototype process which was followed in the pharmaceutical industry for quantification of chemical substances. Therefore, an effort has been taken to develop a simple, rapid and highly sensitive method for the analysis of Cox 2 Inhibitor drug Ketorolac by QbD approach using RP-HPLC method. The optimization phase was done by using QbD technique together with response surface methodology and desirability function.The Chromatographic separation was performed for Ketorolac Tromethamine drug using Phenomenex C18 150 x 4.6 mm,5□ column using Acetonitrile and Phosphate buffer pH 3 (60:40% v/v), detection wavelength is 324 nm and run time is 5.9 min. Results found that Ketorolac Tromethamine shown normal co-ordinates in design space which conducted for negligible interference. The statistical data proved that the method was reliable and reproducible. The method was new, stability indicating, simple, specific, rapid, robust, accurate, and precise for analysis of NSAID- Ketorolac by RP-HPLC.

Keywords: RP-HPLC, Quality by Design (QbD), Ketorolac, Ammonium acetate buffer.

1. INTRODUCTION

The first step in establishing an HPLC method is always to read the literature which includes the solubility profile, the analytical profile, and the stability profile.

For a developed method in pharmaceutical analysis, proper validation of the analytical method is crucial since the analytical methods are crucial for assuring the ongoing efficacy and safety of the goods created in each batch and entirely depends on the determination of quality. [1] Choosing the best column, mobile phase, and detection wavelength can make all the difference when creating HPLC methods for routine analysis. [2, 3]

Method development should ideally follow the phases as- Details of sample, Elucidate separation goals, Requirement of HPLC Procedure and sample pre-treatment, Select detector and detector setting, Select LC method: Preliminary run; evaluate ideal separation condition, Optimize separation conditions-a) Scrutinize the error or need of specific procedure- Retrieve Purified Material b)Quantitative Calibration c)Qualitative Method and Validation of method[4]

Validation is a systematic way to identifying, measuring, evaluating, and documenting.[5]The ICH and FDA guidelines provide a framework for performing validation for pharmacological procedures. ICH recommendations Q2 (R1) are summarizes the typical validation. [6]

This thesis verifies the effectiveness of analytical methods based on RP-HPLC, HPTLC, and LCMS/MS for determining the chosen drug candidates from their formulations, which are present either in a combined form (Fixed dose combination) or from a single active ingredient. This approach is known as Quality-by-Design, and it applies statistical experimental design to demonstrate its viability. To determine how controllable elements interact, the following designs were used: Central Composite Design, Box-Behnken Design, and Taguchi Orthogonal Array Design. The factors utilized at different levels that have the most significant effects on the replies that were chosen were determined based on the results, and they were statistically assessed to determine the best point for the required criteria. By blocking and altering particular parameters, a flowchart will show all the processes of the AQbD technique.

The systematic evolution of a technique through unquestionable decision-making acquired from surveying and evaluating methods is stressed by the AQbD application in method advancements that have been reported to date.[7,8]

Drug manufacturers will be able to investigate the controllable, correctable factors that affect the success rate of process outcomes (in this case, validated method) through the use of AQbD. [8].

The traditional approach to method development starts by keeping one factor constant and changing the other one at a time. This is a non-statistical method known as a one- factor-at-a-time (OFAT) experiment that identifies how the response is impacted by each component. [9,10] The design of experiments, often known as DoE, is a crucial component of AQbD combining the response surface methodology (Box and Draper, 1987). The ICH guideline Q8 (R2) describes the Design-of-Experiments (DoE) as "A systematic, organised strategy for determining the relationship between elements affecting a process and the outcome of that process." [8] The aim of the present work is to change from the quality by testing (Qbt) prototype process which was followed in the pharmaceutical industry for quantification of chemical substances. Therefore an effort has been taken to develop a simple, rapid and highly sensitive method for the analysis of Cox 2 Inhibitor drug by QbD approach using RP-HPLC method. The optimization phase is done by using QbD technique together with response surface methodology and desirability function.[10]

MATERIALS AND METHODS

Part IA:Analytical method development, validation and Force degradation study of Ketorolac by using RP-HPLC method

Equipment and materials

Chemicals and reagents

Ketorolac working standard was received as gift samples from Pratima Pharmaceuticals, Shikrapur, Pune, India. All chemicals, reagents used were of HPLC grade. The tablet -Ketonic) was procured from the local market.

Instrumentation

Thermo 2080 system, P4000 Quaternary pump, UV 6000 PDA detector. The Chromatographic separation was performed using Phenomenex C18 150 x 4.6 mm, 5□ column, detection wavelength is 324 nm and run time is 5.9 min.

Chromatographic conditions

During these investigations a mobile phase was composed Acetonitrile and Phosphate buffer pH 3 in the proportion of 60:40 % v/v gave the best outcomes. During the course of these studies the injection

volume, mobile phase flow rate and analytical wavelength was kept constant (20µL, 1.0 ml/min and 324 nm) for the above selected respectively.

Preparation of standard stock solution:

10mg of Ketorolac was precisely gauged and moved in 100 ml volumetric flask and the content in the flask was dissolved in Acetonitrile.This standard stock solution was further diluted with mobile phase to get 100µg/ml of Ketorolac. The chromatogram for standard drug was shown in Figure no. 1.

Preparation of sample solution:

Powder comparable to 10mg of Ketorolac was taken in 100ml volumetric flask and broke up in 75ml of Acetonitrile with shaking for 5-10 minutes and afterward sonicated.The supernatant liquid was filtered through 0.2µm membrane filter and then transferred into 100 ml volumetric flask and volume was made up with Acetonitrile.

Preparation of Mobile Phase:

Preparation of Phosphate buffer pH 3: Dissolve 1.36 g of potassium dihydrogen orthophosphate and 2 ml of triethylamine in 800 ml water, and adjust the pH to 3.0 with orthophosphoric acid and add sufficient water to produce 1000 ml. Mobile phase prepared by adding Acetonitrile and Phosphate buffer pH 3 in the ratio of 60:40 % v/v.

Procedure for preparation of blank solution:

Acetonitrile and Phosphate buffer pH 3 in the proportion of 60:40 % v/v was used as blank solution.

Method validation parameters of Ketorolac[11,12]

Linearity is a validation parameter used to describe the calibration curve plotted between concentrations versus peak response. The concentration range at which the working standard drug under estimation obeys Beer-Lambert's law and linearity is obtained. The correlation coefficient value should not be less than 0.999. Linearity was established by injecting five aliquots of concentrations of Ketorolac solutions. From the stock solutionof drug, a series of working standard solutions were prepared in the concentration range 4-20 µg /ml. The results of linearity study were enlisted in the table no.1. The linearity graphs obtained for Ketorolac was shown in figure no.2.

Accuracy:[13]

Accuracy of the method was established by standard addition method. A known amount of sample solution (10 µg/ml of Ketorolac) was spiked with 80%, 100% and 120% concentrations of standard solution. The solutions were injected three times (n=3). The assay value of Ketorolac was represented in the table no.2.

Precision

The optimized concentration (10µg/ml of Ketorolac) of mixed standard solution and sample solution was injected six times in to RP-HPLC system. The system precision and method precision values were presented in table no. 3, table no. 4 and table no. 5.

Robustness

Robustness of the developed method was determined by changing the specific chromatographic conditions like flow rate (±5%) i.e. 0.8ml/min and 1.2 ml/min,temperature (28˚C and 32˚C) and mobile phase ratio. The robustness values were presented in table no. 6.

System suitability[14]

Freshly prepared standard solution of Ketorolac was injected six times into RP-HPLC system and the peak areas, retention time, tailing factor, plate count and resolution were determined and the results were presented in table no. 7.

Assay

The percentage purity of the drug content in the pharmaceutical tablet dosage form was performed as per official procedure.

Part IB: QbD-Driven RP-HPLC Method Development, Validation and Estimationof Ketorolac in Dosage Form with Improved Performance

Software based method development[15]

A new Reverse Phase-HPLC strategy was created for Ketorolac by utilizing QbD approach. A Quality by Design with Design of Experiments approach to the development of an analytical method mainly involves two phases as follows:

Screening Phase

In QbD method development, the first stage is process design parameters in that Critical Process Parameters (CPP) or Critical Method Parameters (CMPs) and Critical Quality Attributes (CQAs) performed. By screening phase, once the method developed, screening phase was utilizing Design Expert 9 programming in that Box-Behnken measurable screening configuration was utilized to upgrade the Critical Process Parameters (CPP) or Critical Method Parameters (CMPs) and to assess association impacts of these boundaries on the Critical Quality Attributes (CQAs).This Screening Phase includes the following steps:

Selection of critical method parameters

It should be depends upon what information gain at the time of method development it has been corresponding to CQA. The Critical Method Parameters chosen for the examination are Buffer pH, Organic Phase (% acetonitrile) and Organic Modifier (Methanol).

Selection of Critical Quality Attributes (CQAs)

Generally Critical Quality Attributes associated with drug products, intermediates, excipients, process of drug. It is defined physical, chemical, microbiological properties should be obtained within range as per guidelines. All of these factors interfering in to retention time and tailing factor at the time of method development. The Critical Quality Attributes selected for the study is Retention time and Tailing Factor. These responses were monitored during the experimental trials.

Experimental Trials[16]

As per the Box-Behnken statistical screening design, low, medium and high levels of the critical method parameters were selected based on the preliminary experimentation. So, the Design summary for Box- Behnken screening design is given in table 11. These 12 experimental trials were carried out by using the chromatographic conditions using the previously selected ChrompackC**18** column.

Statistical Analysis and Final Optimization[16,17]

3D response surface plots and Graph plotted after carrying out above trials in to design expert software. These plots depend upon critical method parameters on selected critical quality attributes. After these trials we decided method parameter which is most acceptable and after this observation finalized optimum chromatographic condition. In statistical analysis, ANOVA tool used to determine significance of each parameter

Validation of the optimized method[18]

Validation of analytical procedures was performed for Ketorolac using the following parameters:

System suitability

In this test number of theoretical plates, retention time, and tailing factors were evaluated by injecting 6 replicates of 10µg/ml standard Ketorolac solution.

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

The LOD and LOQ of the developed method determined by injecting low concentration solution of drug in RP-HPLC this was done until a signal to noise ratio of NLT 3:1 and NLT 10:1 was maintained for LOD and LOQ respectively.

Specificity

The interference of other peaks and peak purity was studied by using specificity parameters by recording chromatogram of standard stock solution of Ketorolac (10µg/ml) and blank chromatogram (only diluents).specificity parameters used to study interference of others it may show in retention time.

Linearity and Range[19]

Selected concentrations range solution i.e. 5µg/ml (50%), 8µg/ml (80%), 10µg/ml (100%), 12µg/ml (120%) and 15µg/ml (150%) was prepared from standard solution to perform linearity. Then each level was injected six times into HPLC, chromatograms and recorded all peaks area and chromatograms.

Precision and Accuracy

Precision is reported in terms how it is precise in the different condition i.e.Intraday and interday.3 different concentration solution was prepared (50%, 100% and 150% of the working level).the precision study carried out in different level on the same day and on 3 different days respectively.By using Interday concentration solution performed accuracy.

Analysis of marketed formulation

Accurately weighed fine Ketorolac and transferred into a 10 ml volumetric flask and sonicated for 20 min with 7 ml of Distilled water (diluent). The resulting solution was filtered by using whatman 1 filter paper final solution diluted up to 10 ml with diluents to obtain a final concentration of 10µg/ml. Plotted chromatogram for analysis of formulation. Graph of peak purity is shown in Figure 34.

RESULT AND DISCUSSION

Part IA: Analytical method development, validation and Force degradation study ofKetorolac Tromethamine by using RP-HPLC method

Method Optimization

Many trials were conducted for the optimization of the method for the simultaneous estimation of Ketorolac Tromethamine tablet by changing ratio of buffer and solvents. The optimized chromatographic conditions were presented in table no. 1.

Table No. 1: Optimized chromatographic conditions for Ketorolac Tromethamine

Sr. No	Parameters	Conditions
1.	Column	C18 column 150 x 4.6 mm x 5µ
2.	Flow Rate	1.0ml/min
3.	Mobile Phase Ratio	Acetonitrile and Phosphate buffer pH 3 in the ratio (60:40 % v/v)

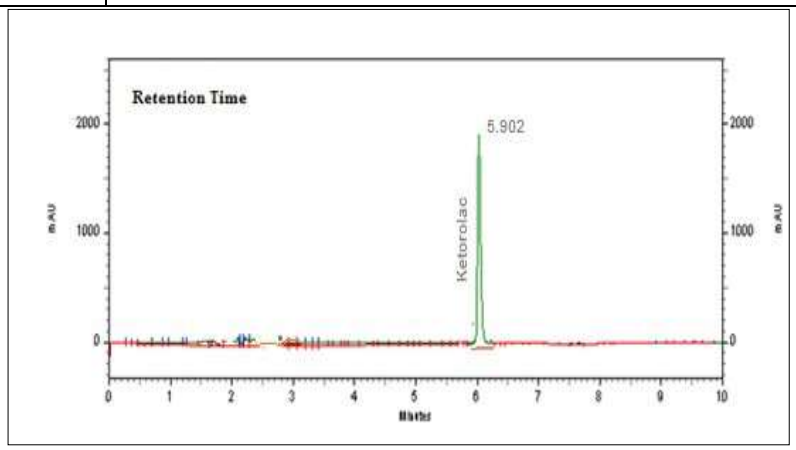


Figure No. 1.: Optimized RP-HPLC chromatogram of Ketorolac Tromethamine

Method Validation

System Suitability

Method validation was carried out as per the ICH Q2(R1) guidelines. The results of the validation parameters are mentioned below. System suitability parameters for Ketorolac Tromethamine standard solution is shown in table no. 2.

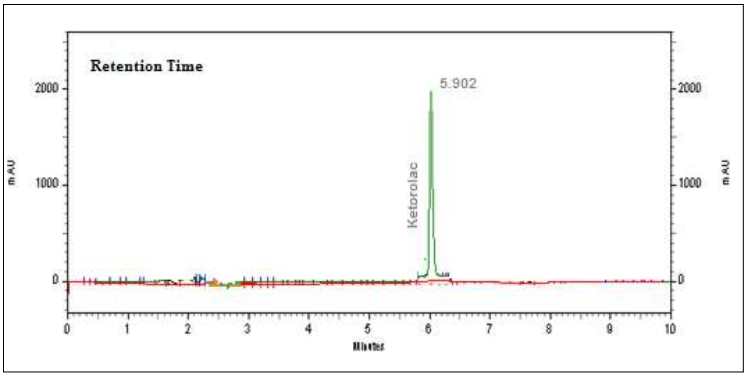
Table No. 2: System Suitability Parameters for Ketorolac Tromethamine

Name of The Compound	Retention Time	Theoretical Plate	Tailing Factor	USP Resolution
Ketorolac Tromethamine	5.902	4695	1.147	-

Specificity:

The RP-HPLC chromatogram of Ketorolac Tromethamine standard solution was shown in figure no. 2. The peaks obtained were sharp and base line separation was clear. The retention time of Ketorolac Tromethamine was found to be 5.902 min.

Figure No. 7.3: RP-HPLC chromatogram of Ketorolac Tromethamine



Linearity:

A standard curve was obtained in the concentration range of 4-20µg/ml for Ketorolac Tromethamine. The slope, intercept and correlation coefficient [r^2] of standard curve were plotted and calculated and are given in figure no. 2 & Table no. 3 demonstrating the linearity of the proposed RP-HPLC method. The LOD value Ketorolac Tromethaminewas found to be 0.00246µg/ml.

Table No. 3: Linearity Studies & Curve of Ketorolac Tromethamine

Linearity Study For of Ketorolac Tromethamine	
CONC. µg/ml	**AREA**
4	2185781
8	3975312
12	6361123
16	7687456
20	9970978
slope	7945.5
RSQ(r2)	0.996
LOD	0.00246

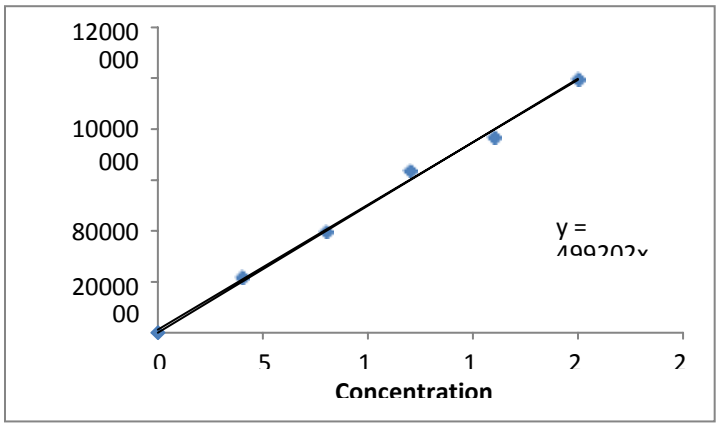


Figure No. 3: Calibration Curve of Ketorolac Tromethamin

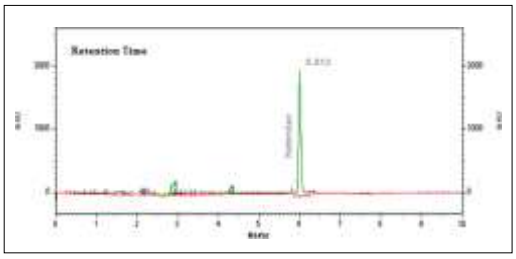


Figure No. 4: Linearity Chromatogram for 4 µg/ml

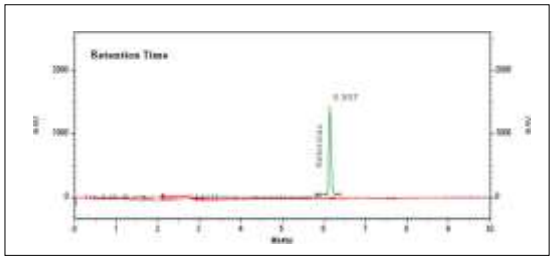


Figure No. 5:Linearity Chromatogram for 8µg/ml

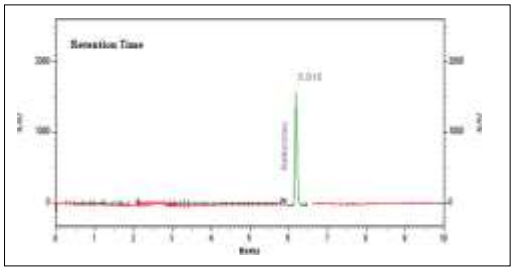


Figure No. 6: Linearity Chromatogram for 12 µg/ml

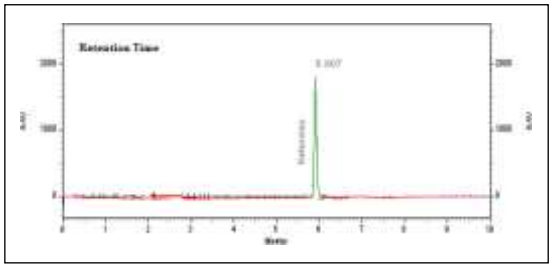


Figure No. 7: Linearity Chromatogram for 16 µg/ml

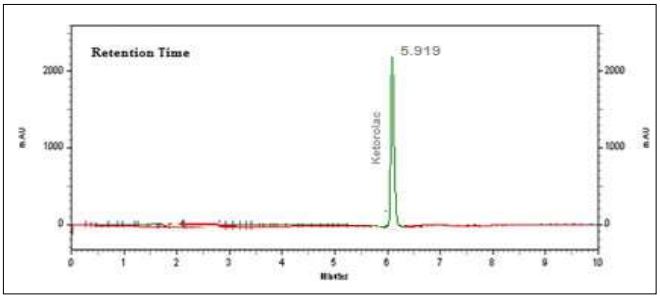


Figure No. 8: Linearity Chromatogram for 20 µg/ml

Precision:

In the present study, precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in sample preparation. The method precision study for six sample preparations in marketed samples showed Figure No. 9 to 15. A RSD of 0.0273% and the 95% confidence interval with the assay range of 99.6-100 for Ketorolac Tromethamine.

For Precision 1

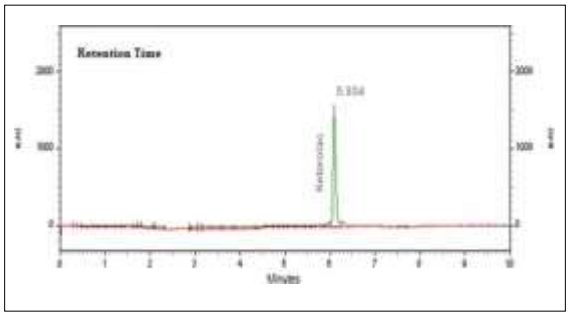


Figure No. 9: Precision chromatogram 1

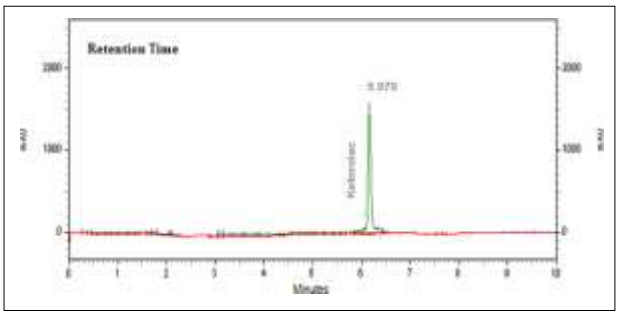


Figure No. 10: Precision chromatogram 2

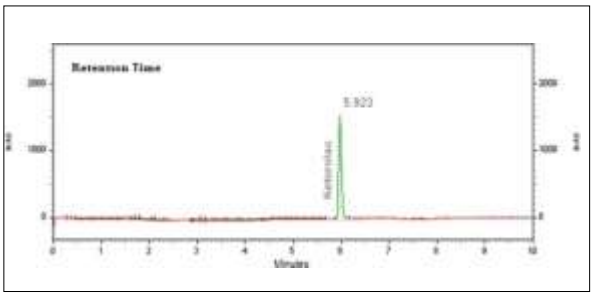


Figure No. 11: Precision chromatogram 3

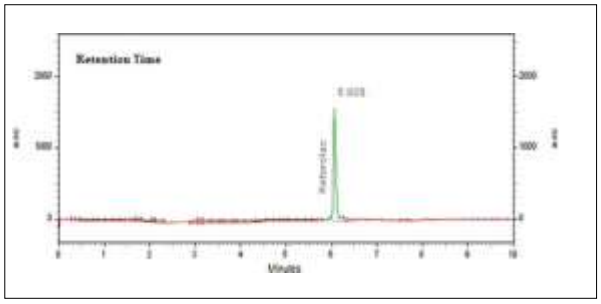


Figure No. 12: Precision chromatogram 4

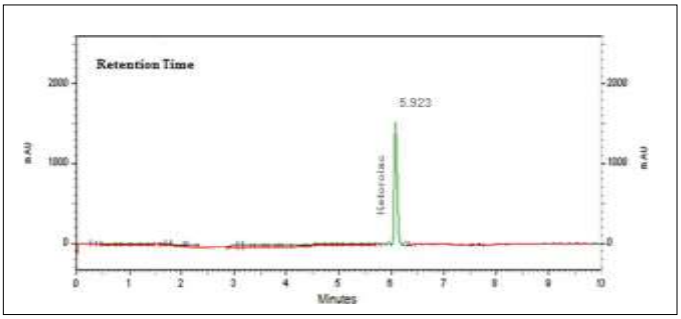


Figure No. 13: Precision chromatogram 5

Table No. 4: Precision 1 study

Sr.No.	Retention Time	Peak area
1	5.934	6361253
2	5.976	6361269
3	5.923	6361259
4	5.928	6361266
5	5.923	6361268
Average Peak area		6361263

For Precision 2

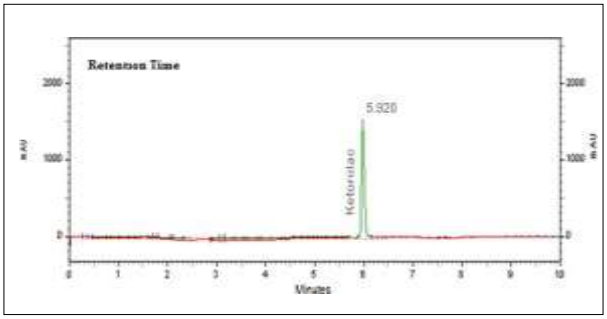


Figure No. 14: Precision chromatogram 1

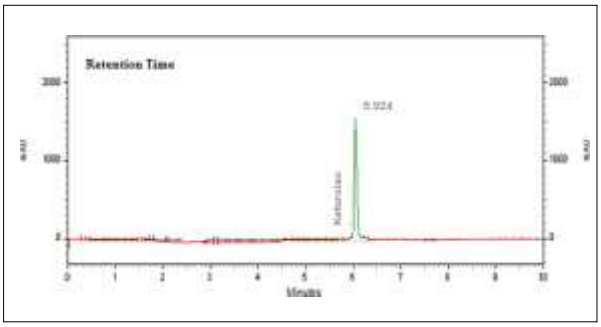


Figure No. 15: Precision chromatogram 2

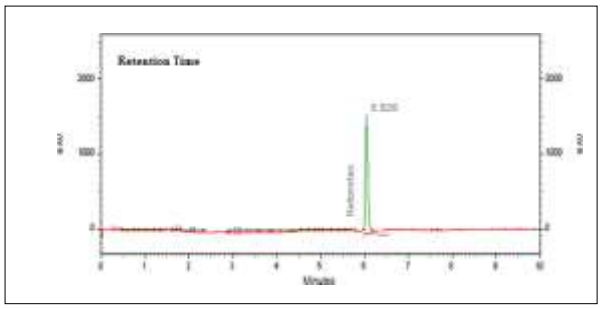


Figure No. 16: Precision chromatogram 3

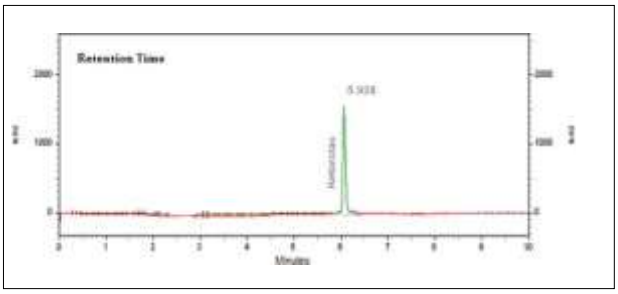


Figure No. 17: Precision chromatogram 4

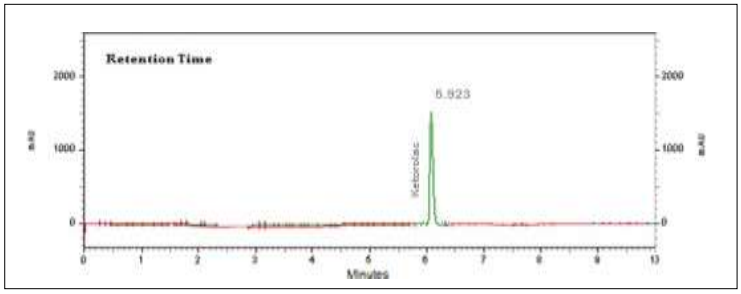


Figure No. 18: Precision chromatogram 5

Table No. 5: Precision 1 study

Sr.No.	Retention Time	Peak area
1	5.934	6361253
2	5.976	6361269
3	5.923	6361259
4	5.928	6361266
5	5.923	6361268
Average Peak area		6361263

For Precision 3

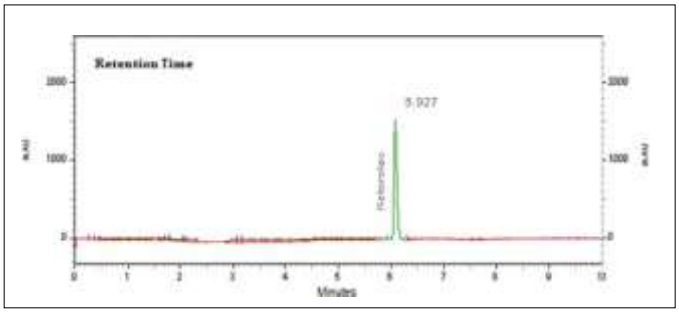


Figure No. 19: Precision chromatogram 1

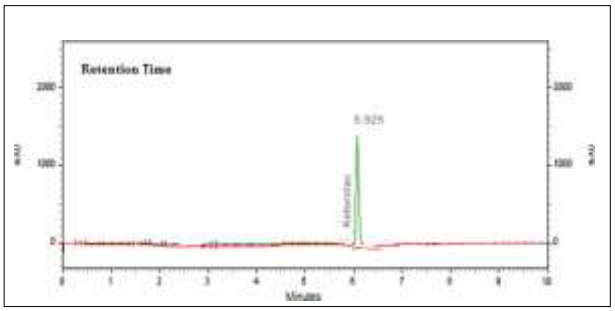


Figure No. 20: Precision chromatogram 2

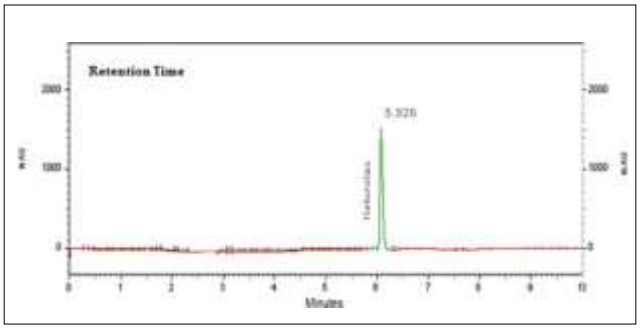


Figure No. 21: Precision chromatogram 3

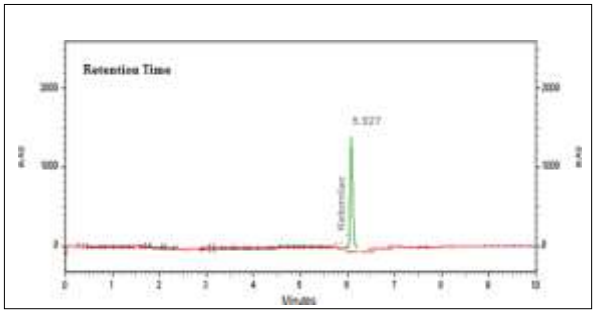


Figure No. 22: Precision chromatogram 4

Figure no. 23: Precision chromatogram 5

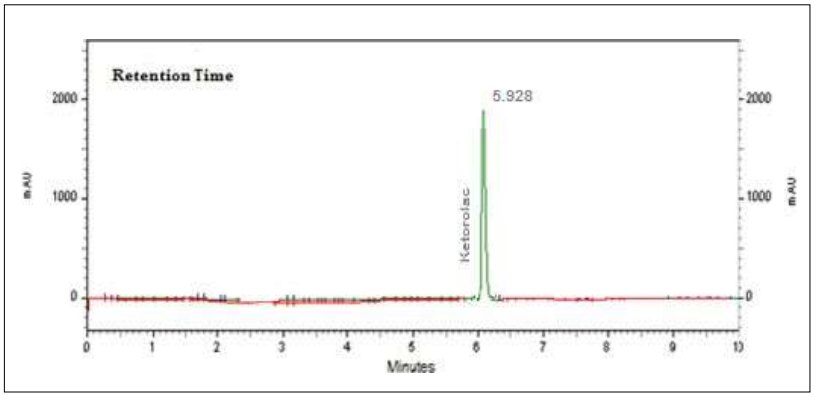


Table No. 6: Precision 3 studies

Sr. No.	Retention Time	Peak area
1	5.927	6361275
2	5.925	6361270
3	5.926	6361286
4	5.927	6361239
5	5.928	6361274
Average Peak area		6361269

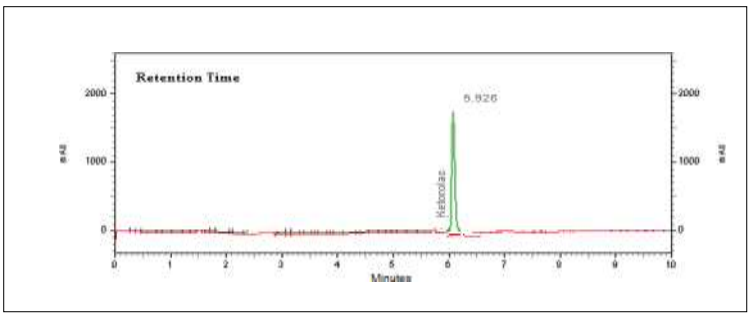


Table No. 7: Precision (Inter and Intraday) Studies for Ketorolac Tromethamine

Method precision (inter and intraday)	
Precision 1	6361263
Precision 2	6361248
Precision 3	6361269
Over all Avg.	6361260
Over all Std Dev.	8.83175383
Over all % RSD	0.000170039

Percentage Relative standard deviation for linearity studies at the time of method development was found to be 0.0001700.

Accuracy:

The accuracy of the present RP-HPLC method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on composite blend collected from formulations of Ketorolac Tromethamine, and were analyzed as per the proposed method. The percentage recoveries were found in the range of 100% with an overall %RSD of 0.487. From the data reported in Table no. 8 that the developed RP-HPLC method was found to be accurate for Ketorolac Tromethamine assay at 100% Accuracy.

Table No. 8: Recovery Studies for Ketorolac Tromethamine by the ProposedMethod

Drug	**Spiked level (%)**	**Amount taken (µg/ml)**	**Amount found (µg/ml)**	**Percent Recovery (% w/w)±RSD**
Ketorolac Tromethamine	80	8	7.88	99.6± 0.456
	100	10	9.99	100.02± 0.487
	120	12	11.97	99.8± 0.434

Robustness Studies:

Robustness is a validation parameter which indicates the developed method resists small but deliberate variations such as flow rate, mobile phase ratio and temperature. The results of robustness study for Ketorolac Tromethamine sample solutions were presented in table no. 7.9. The result shows that the developed method was found to be robust for minute deliberate changes in the mobile phase ratio, temperature and flow rate. The % RSD values were within the limits as per ICH guidelines. The % RSD value of peak areasof Ketorolac Tromethamine was found to be less than 2.

Flow Rate 1

Figure No. 24: Robustness Studies Chromatograms of Ketorolac Tromethamine

Flow Rate 2:

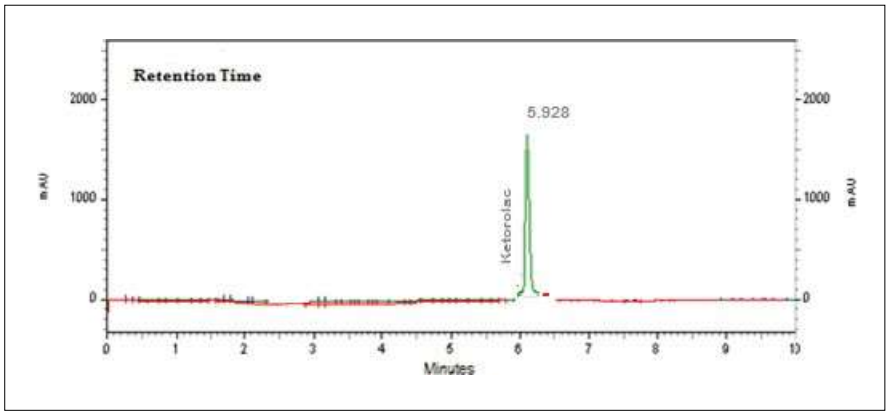


Figure No. 25: Robustness Studies Chromatograms of Ketorolac Tromethamine

Temperature 1:28°C

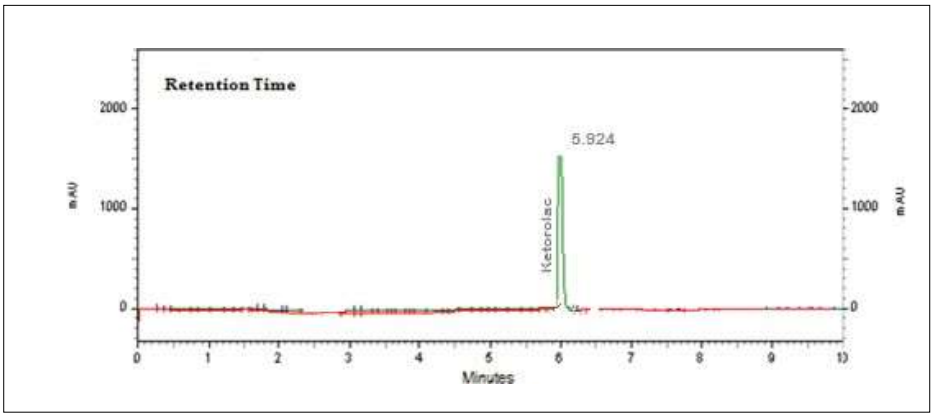


Figure No. 26: Robustness Studies Chromatograms of Ketorolac Tromethamine

Temperature 2:30°C

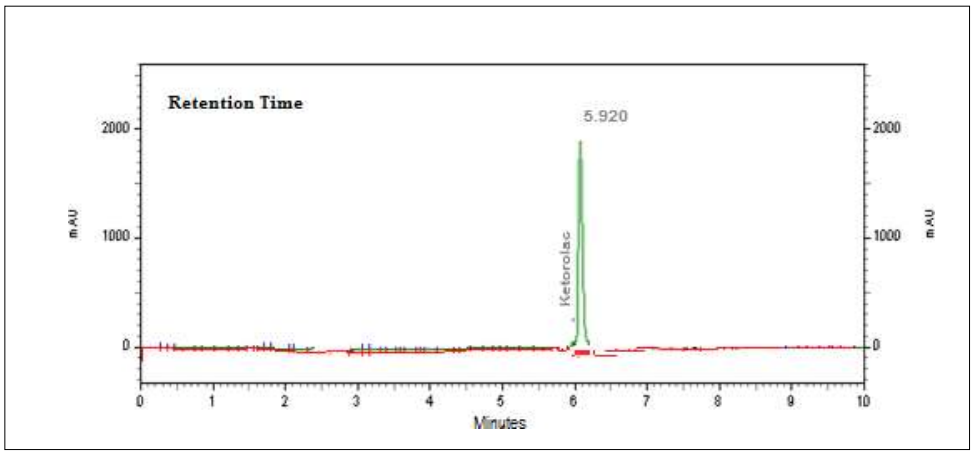


Figure No. 27: Robustness Studies Chromatograms of Ketorolac Tromethamine

Mobile Phase Ratio:

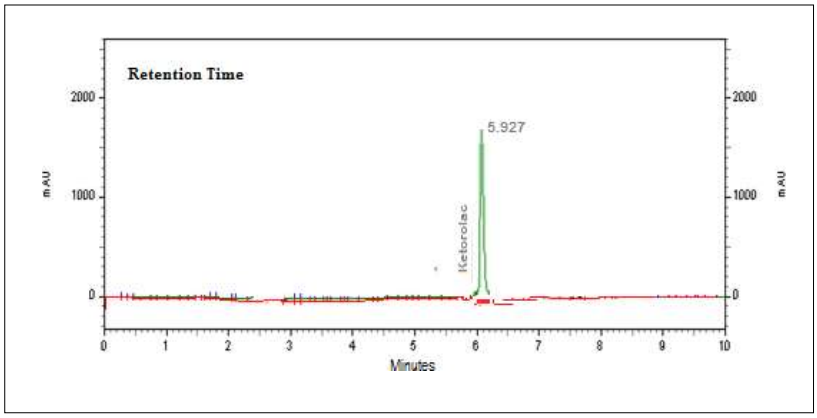


Figure No. 28: Robustness Studies Chromatograms of Ketorolac Tromethamine

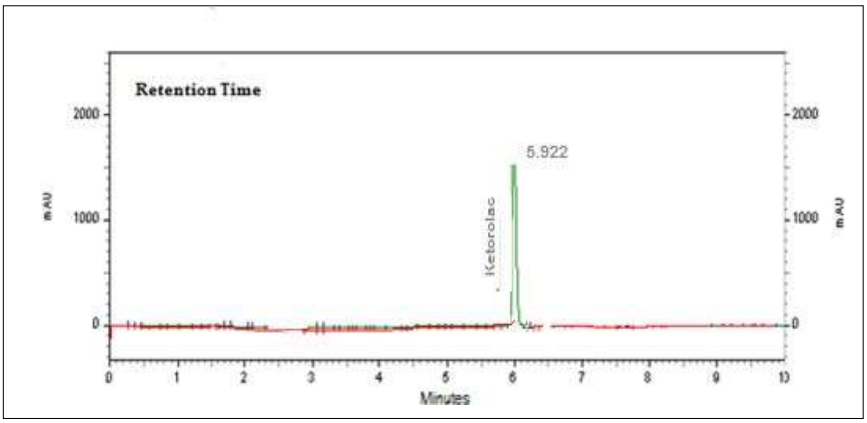


Figure No. 29: Robustness Studies Chromatograms of Ketorolac Tromethamine

Table No. 9: Result of Robustness study of Ketorolac Tromethamine

Sr. No.	Chromatographic conditions	Peak Area	Retention Time
1.	Flow rate (1.0 mL/min)	6361286	5.926
2.	Flow rate (1.2 mL/min)	6361274	5.928
3.	Temperature 28°C	6361255	5.924
4.	Temperature 30°C	6361247	5.920
5.	Acetonitrile :Water 70:30 (v/v)	6361275	5.927
6.	Acetonitrile :Water 65:15 (v/v)	6361256	5.922
7.	Over all Std Dev.		15.003333
8.	Over all % RSD		0.0002

Analysis of Marketed Formulation:

The analysis of marketed formulation for Ketorolac Tromethamine was carried out using optimized HPLC conditions. The total % assay of the proposed method for Ketorolac Tromethamine was found to be 99.94. This showed that the estimation of dosage forms with the proposed RP-HPLC method was accurate and was within the acceptance level of95% to 100% and the results are given in table no. 10.

Table No. 10: Result of Analysis of Marketed Tablets

Drug	Label claim	% Assay	%RSD
Ketorolac Tromethamine(Ketonic)	**10 mg**	**99.94**	**0.24**

Limit of detection and Limit of Quantitation:

LOD and LOQ are the terms used to describe the minimum amount of the drug that can be detected and the minimum amount of the drug that can be quantified by HPLC respectively. The formulas used to calculate LOD and LOQ are mentioned below. The LOD value was found to be 0.00266 µg/mL and LOQ values of Ketorolac Tromethamine were found to be 0.03124 µg/ml.

Part IB: QbD-Driven RP-HPLC Method Development, Validation and Estimation of Ketorolac Tromethamine in Dosage Form with Improved Performance

Choice of Column

Selection of column depends upon the nature of drug.

Ketorolac Tromethamine is heterocyclic in nature. Selection of column depends upon mobile phase (polar, non polar solvents) C^8 column gives poor retention of drug Moreover C^{18} column shown hydrophobic property so it preferred for separation of drug. Use of water- acetonitrile and water-methanol lead to poor precision in retention of analyte so this indicates buffer is required in order to control the ionization of analyte.

Screening Phase

This screening phase was carried out using Design Expert 9 software. In this software, Box-Behnken software was chosen to screening studies and to optimize the critical methods parameters wherein all the parameters were varied simultaneously unlike the conventional OFAT (one factor at a time) approach. The responses obtained after carrying out the 12 experimental trial runs under Box-Behnken design were fed back to DOE software result tabulated in Table no. 12.

Table No. 11: Design Summary for screening studies

Critical Method Parameters	Type	Low level	Medium level	High level
Phosphate Buffer pH 3	Numeric	3	4.5	6
Organic Phase (% acetonitrile)	Numeric	10	20	30
Organic Modifier Methanol	Numeric	10	20	30

Table No. 12: Factor screening by Box- Behnken Design

Run	Factor 1: Phosphate Buffer pH 3	Factor 2: Organic Phase (%ACN)	Factor 3: Organic Modifier Methanol	Response 1: Retention Time	Response 2: Tailing Factor
1	6	10	20	7.2	1.3
2	6	20	30	3.6	1.89
3	6	20	10	3.1	1.79

4	3	30	20	2.52	1.95
5	3	20	10	2.2	1.8
6	4.5	30	30	2.7	2.42
7	3	10	20	6.7	1.9
8	4.5	10	30	6.3	2.3
9	6	30	20	2.3	2.19
10	4.5	30	10	2.8	1.99
11	3	20	30	2.5	2.1
12	4.5	10	10	5.9	1.8

Statistical Analysis and Final Optimization

ANOVA is the statistical tool which is used to estimation of procedure such as variance. It is used to analyze difference among means. It is form of statistical hypothesis testing. The result calculated by null hypothesis. The significance value of null hypothesis was defined at $p \geq 0.05$. It indicates variation in all factors which has no influence on the responses. The response surface plots and graph plots evaluated by ANOVA used different method parameters.

The two response variables i.e. Retention time and Tailing factor were statistically evaluated as follows:

Retention time

In critical quality attribute, retention time is one of the most important attributes come under in experimental design. It can evaluate by using different statistical analysis tools and plots which are mentioned below.

Analysis of Variance (ANOVA)

The ANOVA result for retention time is tabulated in Table no. 13. The ANOVA result data reveals that '*p* value' for the model is 0.0055 which indicates this model explains significant variability. Also, the Model F-value of 9.28 implies the model is significant. The "Pred R-Squared" of 0.4979 is in reasonable agreement with the "Adj R- Squared" of 0.6932. Adequate Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 7.304 in the model indicates an adequate signal. Tailing factor ANOVA tabulated in table no. 14.

Moreover, the *p* value for pH and % acetonitrile is 0.0053 and 0.0008 respectively i.e. less than 0.05which indicates that both these parameters have significant effect on Retention time.

Table No. 13: ANOVA Result for retention time

	Model	**Factor 1 :Phosphate buffer pH 3**	**Factor 2: %ACN**	**Factor 3: % Organic Modifier Methanol**
Sum of squares	31.93	40.10	31.13	0.15
Mean squares	10.64	40.10	31.13	0.15
F value	9.28	53.50	27.15	0.13
p Value	0.0055	0.0053	0.0008	0.7258
Std. deviation	1.07	-	-	-
R- squared	0.7769	-	-	-
Adj- R squared	0.6932	-	-	-

Predi- R squared	0.4979	-	-	-
Adequate precision	7.304	-	-	-

Table No. 14: ANOVA Result for tailing factor

	Model	**Factor 1 :pH**	**Factor 2: %ACN**	**Factor 3: % Phosphate buffer pH 3**
Sum of squares	40.23	0.042	0.20	0.22
Mean squares	14.82	0.042	0.20	0.22
F value	7.46	0.72	3.35	8.31
p Value	0.0128	0.4201	0.1044	0.0471
Std. deviation	1.37	-	-	-
R- squared	0.6961	-	-	-
Adj- R squared	0.5510	-	-	-
Predi- R squared	0.3962	-	-	-
Adequate precision	6.237	-	-	-

Graph Plots

The three graph plots indicate the values of retention time at different levels of pH, % organic phase and % methanol. In Figure the early retention time is neglected because there may be chance to merging of eluting analyte peak with solvent peaks hence after 5 min. retention time is considered. The retention time of analyte peak is less than 3 mins at 20% and 30% of organic modifier. Thus, 10% organic phase is suitable as it givesretention time of peak between 5 to 8 mins.

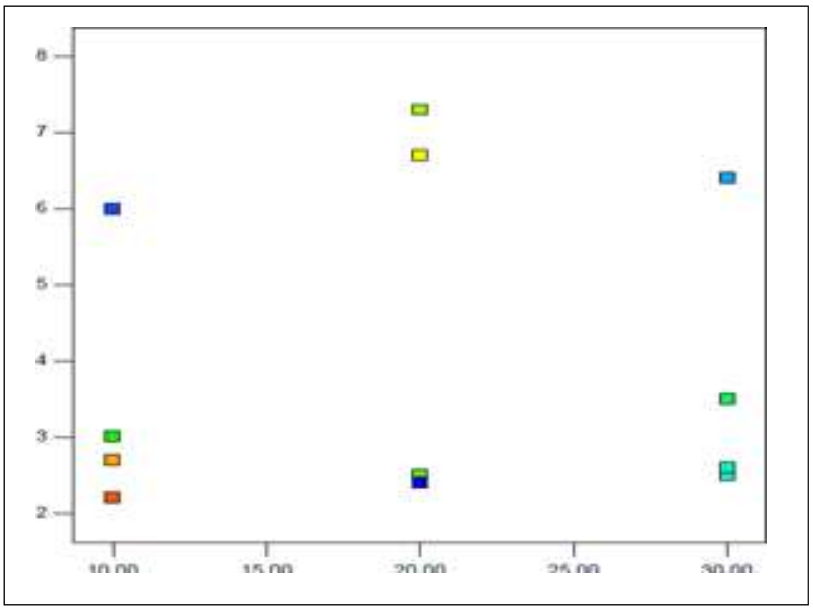


Figure No. 30 : X1: pH, X2: Retention time

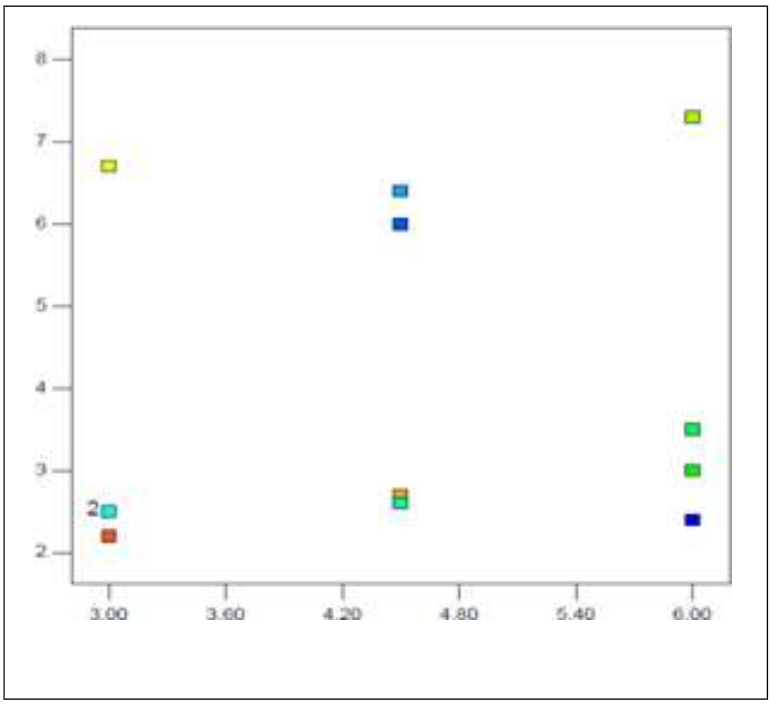


Figure No. 31: X1: Organic phase, X2: Retention time

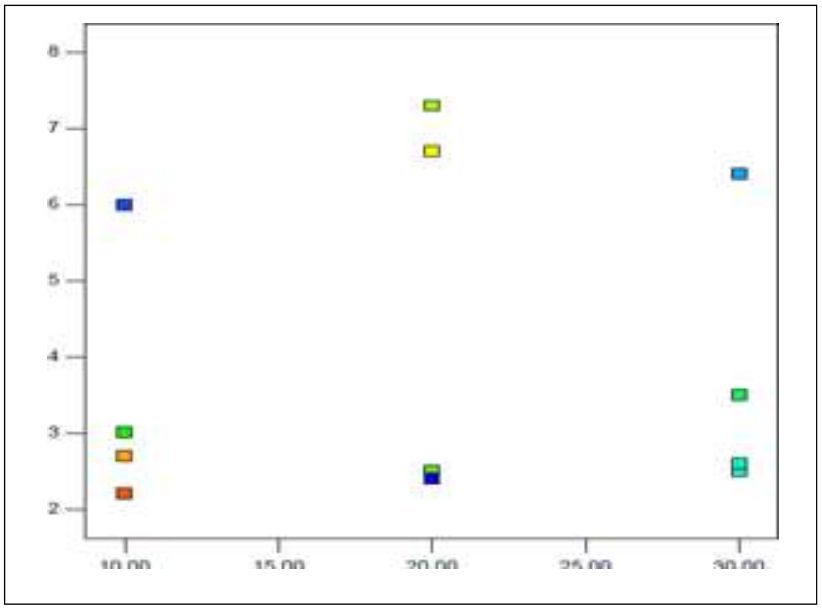


Figure No. 32: X1: Organic modifier, X2: Retention time

3D Response-Surface Graph

The response surface graph is a 3D plot with pH and % organic phase on X and Y axis respectively and retention time on Z axis. As observed in the Fig, The factor % organic modifier is kept constant as pH and %organic phase are most influential parameters of retention time which was evident from the ANOVA result. The above graph plot indicates that the retention time is above 5 mins at pH 6 and

10% organic phase and at pH 3 and 10% organic phase. While, the retention time is less at other data points, due to which these points are not considered.

3D Response-Surface Graphs

The response surface graph is a 3D plot with pH and %organic phase on X and Y axis respectively and tailing factor on Z axis. As observed in the Fig, The factor % organic modifier is kept constant to 20%. The above graph plot indicates that the tailing factor is closer to 1 when pH is 6 and organic phase is 10%. Other data points, however, have tailing factors that are greater than 2, which is outside of acceptable bounds. Thus, the final optimized critical parameters predicted from the above plots are tabulated in Table no. 11.

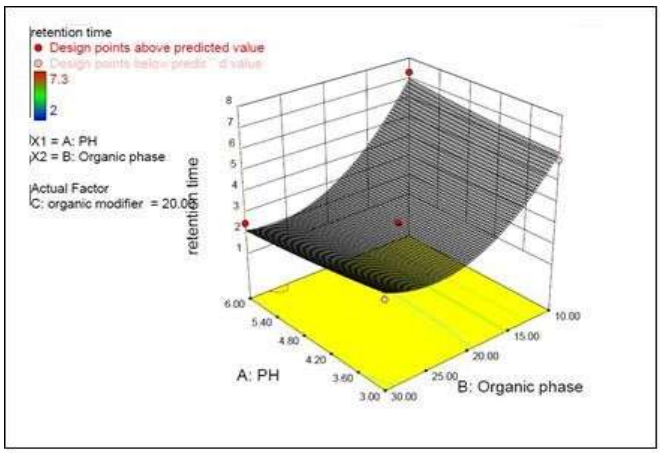


Figure No. 33: 3D Response-Surface Graphs for Retention Time

The predicted result for the response variables estimated with 95% CI was found to be Retention time as 7.21 mins and tailing factor as 1.36.

Final Optimized Chromatographic Conditions

The final chromatographic conditions developed using Quality by Design approach is tabulated in Table No. 15

Table No. 15: Final Optimization Result of the optimized method Validation

Critical Method Parameters	Low level	Medium level	High level	Final Level selected
Phosphate Buffer pH 3	3	4.5	6	6
Organic Phase (% Acetonitrile)	10	20	30	10
Organic Modifier (Methanol)	10	20	30	20

System suitability, Specificity, Limit of Detection, Limit of Detection, Linearity, Range, Accuracy and Precision parameters can performed after once the chromatographic conditions were set.

Evaluation of system suitability

After injecting standard solution for six times the %RSD obtained within acceptable range. Tailing factor was less. And theoretical plates found above 2000. Thus all these parameters obtained within range as per guidelines.

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

LOD and LOQ was performed by decreasing concentration level of Ketorolac Tromethamine as long as a signal to noise ratio of Not less than 3:1 and 10:1 is maintained respectively. The LOD and LOQ for Ketorolac Tromethamine were found to be 200ng/ml. and 600ng/ml respectively.

Specificity

After the optimized method Blank (Diluent) and Standard solution (10µg/ml) were injected and the specificity parameter carried for found out interference in peak around the retention time. Even baseline did not show any significant peak. The green part of peak purity graph corresponds to high purity of 97.22%.

Linearity and Range

Linearity was evaluated in the range of 50% to 150% of the working concentration level i.e.,10µg/ml (5µg/ml- 15µg/ml) for Ketorolac Tromethamine. The Linearity was confirmed in the range of 5µg/ml - 15µg/ml. The Co-efficient of Co-relation (R2) was found to be 0.999 and the equation of the line was y = 73636x + 170.3 as evident from the below calibration curve.

Precision and Accuracy

The precision of the method shows that interday and intraday found within acceptance range. The % RSD was found to be satisfactory. The accuracy data shows that the % mean recovery of Ketorolac Tromethamine at each level is within the acceptance criteria of 98.0% - 102.0%.

Stability Indicating Assay of Ketorolac Tromethamine

The force degradation study was performed for stability of Ketorolac Tromethamine. It is indicated that the drug was degraded by 4.595%, 10.326% and 25.497% when subjected to acid hydrolysis, base hydrolysis and oxidation degradation respectively. This shows that there is not found any impurity. Drug was found to be stable in different degradation conditions like acid hydrolysis, base hydrolysis and oxidation degradation thermal, degradation and photolytic degradation.

Application on Marketed Formulation

The developed Stability Indicating RP-HPLC method was successfully applied for the estimation of Ketorolac Tromethamine from the marketed formulation which was found to contain 98.92 % of the Label Claim.

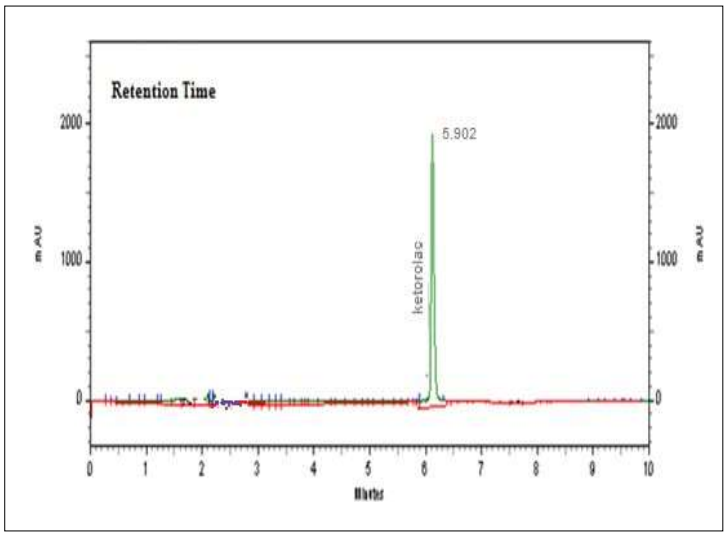


Figure No. 34: Chromatogram of sample run

Conclusion:

QbD strategy was adopted to develop a robust and efficient RP-HPLC method for the analysis of Non-steroidal Anti-inflammatory Drug (NSAIDs)Ketorolac Tromethamine. The proposed RP-HPLC method for Ketorolac Tromethamine [Non-steroidal Anti- inflammatory Drugs (NSAIDs)] was validated as per ICH Q2 (R1) guidelines.

Design space by QbD method of analysis has been carried out for Ketorolac Tromethamine using ICH 2 (B) guidelines. Optimization data analysis and model validation were carried out using Design Expert® ver. 11 software to fit the experimental data into the second degree quadratic polynomial models for estimating various responses, along with the inspected variables. The models that were employed for the screening and optimization processes validated the method's predictability and were highly significant.

Results found that Ketorolac Tromethamine shown normal co-ordinates in design space which conducted for negligible interference. The statistical data proved that the methods were reliable and reproducible. The methods were new, stability indicating, simple, specific, rapid, robust, accurate, and precise for analysis of NSAID Ketorolac Tromethamine by RP- HPLC.

References:

1. Ahuja S, Scypinski S. Modern Pharmaceutical Analysis: An Overview. Handbook of Modern Pharmaceutical Analysis. Academic Press; 2001:243.

2. Patil, M.P.N., 2017. HPLC Method Development–A Review. Journal of pharmaceutical Research and education,1 (2),Pp.243-260.

3. Zotou, A., 2012. An overview of recent advances in HPLC instrumentation. Central European Journal of Chemistry, 10(3), Pp.554-569.

4. Snyder LR, Kirkland J, Glajch J. Practical HPLC Method Development,2nd ed, New York:John Wiley & Sonsinc; 1997.

5. Chan, C.C., 2011.Principles and practices of analytical method validation: Validation of Analytical Methods Is Time- Consuming but Essential. The quality assurance Journal, 14(3-4), Pp.61-64.

6. Guideline, I.H.T., 2005. Validation of analytical Procedures: Textandmethodology.Q2 (R1), 1(20), P.05.

7. Garg LK, Sait SS, Krishnamurthy T, Kumar CHRP. Quality by Design (Qbd): A Practical Experimental Design Approach by Blocking and Varying Certain Factors of a Stability-Indicating HPLC Method for Simultaneous Determination Of Omeprazole And Ketoprofen. Journal of Liquid Chromatography & Related Technologies. 2015; 38(6):677-86.
8. Vogt FG, Kord AS. Development of Quality-By-Design Analytical Methods. Journal of Pharmaceutical Sciences. 2011; 100(3):797-812.
9. Bergum Dklaj. Application of Quality by Design (Qbd) To the Development and Validation of Analytical Methods. In: Riley CM, Rosanske TW, Riley SRR, Editors. Specification of Drug Substances And Products: Development And Validation of Analytical Methods. 3: Newnes; 2013. P. 29-73.
10. Peraman R, Bhadraya K, Padmanabha Reddy Y. Analytical Quality by Design: A Tool for Regulatory Flexibility and Robust Analytics. International Journal Of Analytical Chemistry. 2015; 2015:9.
11. Izraelevitz AM, Anderson-Cook CM, Hamada MS. Illustrating The Use Of Statistical Experimental Design And Analysis For Multiresponse Prediction And Optimization. Quality Engineering. 2011; 23(3):265-77.
12. Guidance for Industry, "Q8 (R2) Pharmaceutical Development" (Nov 2009) Http://Www.Fda.Gov/Downloads/Drugs/Guidance compliance regulatoryinformation/Guidances/Ucm073507.Pdf. 2009.
13. Jenke, D.R., 1996. Chromatographic Method Validation: A Review of Current Practices and Procedures. II. Guidelines for Primary Validation Parameters. Journal of Liquid Chromatography & Related Technologies, 19(5), Pp.737-757.
14. Jenke, D.R., 1996. Chromatographic Method Validation: A Review of Current Practices and Procedures. II. Guidelines for Primary Validation Parameters. Journal of Liquid Chromatography & Related Technologies, 19(5), Pp.737-757.
15. Hartan, M. And Tambe, S.R., 2022. Implementation of Qbd Approach to The Analytical Method Development And Validation For The Estimation Of Propafenone Hydrochloride In Tablet Dosage Form. Chromatography Research International, 2022.
16. Fukuda, I.M., Pinto, C.F.F., Moreira, C.D.S., Saviano, A.M. And Lourenço, F.R., 2018. Design Of Experiments (Doe) Applied To Pharmaceutical And Analytical Quality By Design (Qbd). Brazilian Journal Of Pharmaceutical Sciences, 54.
17. Nagarajan, N.C., Sahini, K., Vinod, S., Thajnisha, J., Santhosh, C.G., Pavithra, J. And Thiyagarajan, S., 2022. Response Surface Methodology For The Optimized And Validated Investigation Of Ropivacaine In Bulk And Tablet Dosage Form By RP–HPLC.
18. Sha'at, M., Spac, A.F., Stoleriu, I., Bujor, A., Cretan, M.S., Hartan, M. And Ochiuz, L., 2022. Implementation Of Qbd Approach To The Analytical Method Development And Validation For The Estimation Of Metformin Hydrochloride In Tablet Dosage Forms By HPLC. Pharmaceutics, 14(6), P.1187.
19. Basso, J., Mendes, M., Cova, T.F., Sousa, J.J., Pais, A.A. And Vitorino, C., 2018. Analytical Quality by Design (Aqbd) As a Multiaddressable Platform For Co-Encapsulating Drug Assays. Analytical Methods, 10(47), Pp.5659-5671.