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Application of Quality by Design Approach for Development and Validation of Stability Indicating RP-HPLC Method for Rilpivirine Hydrochloride in Bulk

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ABSTRACT

The present study describes the implementation of Quality by Design approach to the development and validation of stability indicating RP-HPLC method for Rilpivirine HCl. Optimization was done by response surface methodology, applying a three-level Box-Behnken design. Three factors selected were the Concentration of Methanol and Water (mobile phase), Flow Rate, pH. The developed chromatographic method was validated concerning the ICH Q2 (R1) guidelines for linearity, precision, range, accuracy, LOD, and LOQ. The maximum Absorbance of the drug (λ_{max}) was found to be 305 nm. The optimized method consists of mobile phase methanol: water (pH 5.2) (85: 15), and a flow rate of 0.8 ml/min, which was optimized by using design expert software. The linearity of the developed method was established over the concentration range of 20-100 µg/ml for Rilpivirine HCl with a correlation coefficient (R^2) of 0.999. The percent RSD for accuracy and precision of the method was found to be less than 2%. The limit of quantitation (LOQ) 1.04µg/ml and limit of detection (LOD) of 3.16µg/ml are relatively low to permit the determination of low concentrations of the drug. Stability (Forced Degradation) studies were accomplished in various conditions like acidic, alkali, oxidation, thermal and photolytic.

INTRODUCTION

The pharmaceutical industry is constantly in search of new techniques to ensure and enhance product quality in terms of its safety, quality, and efficacy. However, still, problems with drug recall, manufacturing failure cost, scale-up issues and regulatory burden in the recent past produces a huge challenge for the industry. In the traditional approach, the product quality and performance are predominantly ensured by product testing, with limited understanding of the process and critical process parameters. Regulatory bodies are therefore focusing on implementing Quality by Design [QbD], a more precise and science-based approach that improves process understanding by reducing process variation and enabling process-control strategies.

QbD approach helps to deal with quality issues efficiently by analyzing problems and their root cause. It comprises identifying all critical attributes including process parameters and material attributes (Figure 01). These parameters help in getting better process understanding thereby resulting in the development of a robust process with the least errors. This leads to identifying a design space where all the parameters could result in quality products. Changes in any such parameters during development and lifecycle management should be looked upon as opportunities to gain additional knowledge and further support the establishment of the design space. Design space is proposed by the applicant and is subject to regulatory assessment and approval. Working within the design space is not considered as a change. Movement out of the design space is a change and would normally initiate a regulatory post approval change process. [1-2]

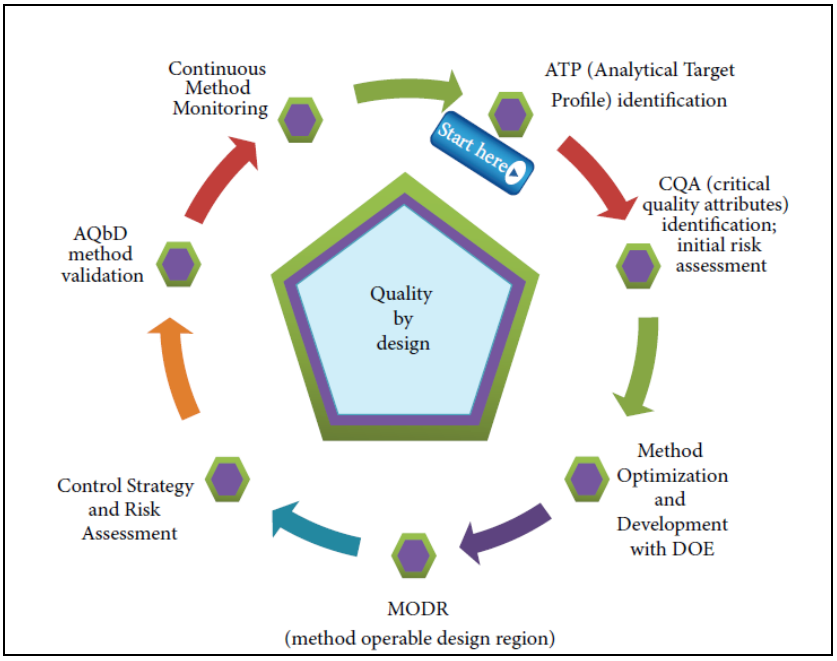


Figure 01: Features of QbD

In QbD-

- The product is designed to meet Quality.
- The process is designed to consistently meet all critical quality attributes.
- The impact of all material and process attributes on final product quality is understood.
- The process is consistently evaluated and updated to meet quality.
- Critical sources of variability are identified and controlled.
- Validated control strategies are developed.

Elements of QbD in Analytical Method

a. Analytical Target Profile (ATP)

b. Critical Quality Attribute (CQA)

c. Method Design

d. Critical Process Parameters (CPP)

e. Risk Assessment

f. Design Space (DS)

g. Method Operable Design Region (MODR)

h. Design of Experiment (DoE)

i. Control Strategy (CS)

j. Process Analytical Technology (PAT)

k. Continuous Method Performance

Rilpivirine is a second-generation non-nucleoside reverse transcriptase inhibitor (NNRTI) with higher potency, longer half-life, and reduced side-effect profile compared with older NNRTIs, such as efavirenz. It is a diarylpyrimidine, a class of molecules that resemble pyrimidine nucleotides found in DNA. It binds to reverse transcriptase which results in a block in RNA and DNA- dependent DNA polymerase activities. Not a single QbD study is published for stability-indicating RP-HPLC method for estimation of rilpivirine and very few methods were published for the estimation of rilpivirine involving HPLC techniques and spectrophotometric methods. Very few HPLC techniques available in the literature discussed the stability-indicating Method development and validation. Hence we attempted to develop and validate a stability-indicating method for the estimation of rilpivirine by application of Quality by Design approach. [3]

This analytical method aims to separate and quantify the main compound while abiding by the method performance benchmark based on regulatory desideratum's, such as linearity, accuracy, precision, robustness. Another aim is to develop an analytical method by using cost-effective mobile phases such as methanol and water.

The paramount objective of this study is to execute a QbD strategy to develop and validate an RP-HPLC method to build-in the quality in the course of method development to secure optimum method performance over the lifetime of the product.

During the literature survey, it was found that not a single study of method development by application of quality by design for Rilpivirine HCl is established even though few stability-indicating types of research were found. Chilukuri M *et al*. studied the Degradation Pathway

for Rilpivirine Hydrochloride by Validated Stability Indicating UP-LC Method with Mobile Phase Acetonitrile: 0.1M ammonium acetate buffer pH4 (50:50 v/v) and λ_{max}: 295 nm[4]. Thota S*et al*. studied Estimation of Rilpivirine in bulk and pharmaceutical dosage form with Mobile Phase. Acetonitrile: potassium dihydrogen phosphate buffer(40/60 v/v) pH 2.8 and λmax: 282 nm[5]. Ghosh S *et al*. studied Method development and validation of Rilpivirine in bulk and Tablet doses form by RP-HPLC method with Mobile phase: Acetonitrile: Phosphate buffer (60:40 v/v) and λ_{max}: 282 nm[6].

Reddiah V *et al*. studied the Effective estimation of Rilpivirine by HPLC method in tablet dosage forms and it's in vitro dissolution assessment with Acetonitrile: Ammonium acetate buffer (55:45 v/v) and λ_{max}: 280 nm[7]. Dr. Yashoda *et al*.studied RP-HPLC method development and validation of Rilpivirine with Acetonitrile: Acetate buffer (pH=4.0) = 65:35 (v/v) and λ_{max}: 260 nm[8].

The optimization was done by using only Methanol and Water as a mobile phase that made our method cost-effective as relative to other methods found during the literature survey.

MATERIALS AND METHODS:

Following table 01 is the list of reagents and chemicals used.

Table 01: List of Reagents and Chemicals Used.

Sr. No.	Reagents and Chemicals	Make	Details
1	Water	MI	HPLC grade
2	Methanol	Finar	HPLC grade
3	Triethylamine	Molychem	AR grade
4	O-Phosphoric acid	Finchem	AR grade

Selection of mobile phase:

Following table 02 shows number of trail runs for method development.

Table 02: Rilpivirine Hydrochloride RP-HPLC method development trials

Sr. No.	Mobile Phase	Column	λ max	Flow Rate	Inference of Method
1	Methanol: Water (90:10) pH 5	Chemsil C_{18}	305nm	1ml/min	Rejected
2	Methanol: Water (85:15) pH 5	Chemsil C_{18}	305nm	1ml/min	Rejected
3	Methanol: Water (75: 25) pH 5	Chemsil C_{18}	305nm	1ml/min	Rejected
4	Methanol: Water (70: 30) pH 5	Chemsil C_{18}	305nm	1ml/min	Rejected
5	Methanol: Water (65: 35) pH 5	Chemsil C_{18}	305nm	1ml/min	Rejected
6	Methanol: Water (60: 40) pH 5	Chemsil C_{18}	305nm	1ml/min	Rejected
7	Methanol: Water (80:20) pH 5	Chemsil C_{18}	305nm	1ml/min	Accepted

Optimized Chromatographic Conditions:

The following table 03 shows chromatographic conditions were established by trial and error and were kept constant throughout the method.

Table 03: Optimized Chromatographic Condition

Parameter/ Conditions	Description/Values
Column name	Chemsil C18, 250 × 4.6mm, 5µ
Detector	305 nm
Flow rate	1ml/min
Injection volume	10µl
Column oven Temperature	Ambient
Retention time	5.1 min
Mobile Phase	Methanol: Water (80:20) pH 5

Standard Stock Solution:

Procedure:

Accurately weighed 25 mg of Rilpivirine Hydrochloride was transferred to a volumetric flask of 25 ml, Add methanol up to mark. Sonicate it to dissolve it completely. The resultant solution is used as the standard stock solution of Rilpivirine Hydrochloride (1000 ppm).

Working stock solution:

Procedure:

Remove 0.5 ml of Stock solution in 10 ml volumetric flask Dilute it with diluent to get 50 ppm solution.

Development of RP-HPLC Method with Design Space and Control Strategy determination by optimization study:

All the calculations for the contemporaneous optimization study and statistical analysis were performed using Design Expert® software (Design Expert trial version 12.0; State-Ease Inc., Minneapolis, MN, USA).

Application of Design of experiments for method optimization

Design of experiments (DOE-1): Thus, 3^3 randomized response surface designs with a Box-Behnken design were used with 17 trial runs to evaluate the effect of three factors on the three key response variables. In this design 3 factors were analyzed, each at 3 levels, and experimental trials were carried out at all possible combinations. The flow rate, pH of Water, mobile phase composition were selected as independent variables and retention time (RT), Theoretical Plate number (TPN) and Asymmetric Factor were selected as dependent variables based on risk analysis. The data resulted was processed into Design Expert 12.0 software and analyzed statistically with the help of analysis of variance (ANOVA). The Translation of coded levels in actual values and probable trial runs using 3^3 Box-Behnken designs are as shown in the table 04.

Table 04: Translation of coded levels in actual values

Level of Variables	Concentration of Factors		
	Flow rate (ml/min)	pH	Mobile Phase Composition (Methanol: Water)
Low level (-1)	0.8	4.8	75:25
Medium level (0)	1.0	5.0	80:20
High level (+1)	1.2	5.2	85:15

System Suitability Test:

The tests were performed by collecting data from five replicate injection of standard drug solution (50 ppm).

Validation of the method for analysis of Rilpivirine Hydrochloride:

Linearity:

Determination:

The linearity of the analytical method is determined by the mathematical treatment of test results obtained by the analysis of samples with analyte concentrations across the claimed range. The area is plotted graphically as a function of analyte concentration.

Preparation of standard stock solution:

25.0 mg of Rilpivirine Hydrochloride working standard was weighed accurately and transferred into a volumetric flask of 25.0 ml; methanol was added and sonicated it to dissolve. From that remove 1 ml in 10ml volumetric flask and dilute it with diluent up the mark. This solution was used to prepare a linearity solution.

Preparation of linearity solution:

Linearity was performed by diluting standard stock solutions. From stock solution aliquots of 2, 4, 6, 8, 10 ml diluted to 10ml with a diluent such that the final concentration of Rilpivirine hydrochloride in the range of 20-100ppm.

Accuracy (by Recovery method)

Preparation of standard stock solution:

25 mg of Rilpivirine Hydrochloride working standard was weighed accurately and transferred into a 25 ml volumetric flask, methanol was added and sonicated to dissolve and finally, the volume was made with diluents and mixed. The working standard concentration is 1000 µg/ml. Remove 1ml and dilute up to 10ml with the mobile phase to give 100 µg/ml solution.

Procedure for Preparation of sample solution:

Prepare the standard solution by taking stock solution equivalent to 50%, 100%, and 150%, each in triplicate. Inject each preparation into the HPLC system. The table 05 shows the various dilutions for Accuracy.

Table 05: Dilution table for Accuracy

Sample	Stock solution (ppm)	Sample solution (ppm)	Final volume (ml)
Accuracy 50%	50	25	10
Accuracy 100%	50	50	10
Accuracy 150%	50	75	10

Precision:

Determination:

Prepare six different test solutions of 50 ppm test concentration from the same sample matrix of the homogeneous sample.

Preparation of standard stock solution:

25.0 mg of Rilpivirine Hydrochloride was weighed accurately and transferred into 25.0 ml volumetric flask, methanol was added and later sonicated it to dissolve. From that remove 1ml in 10ml volumetric flask and dilute it with diluent up the mark with the mobile phase to give 100µg/ml solution.

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

The lowest concentration of the analyte in the sample that the method can detect but not necessarily quantify under the stated experimental conditions simply indicates that the sample is below or above a certain level.

$$LOD = 3.3 \times \frac{(SD)}{S}$$

Where, **SD** = Standard deviation

S= Slope

$$LOQ = 10 \times \frac{(SD)}{S}$$

Where **SD** = Standard deviation

S= Slope

Forced Degradation Study on Rilpivirine Hydrochloride by RP-HPLC:

Control sample:

An accurately weighed amount of 10 mg of Rilpivirine Hydrochloride was transferred in a clean and dry volumetric flask and 10 ml of diluents were added and sonicated to dissolve it completely and volume made up to the mark with diluents. The solution was passed through the Whatman filter paper. Pipette out 0.5 ml of solution in a volumetric flask of 10 ml and diluted up to the mark with diluent.

Acid hydrolysis:

An accurately weighed amount of 10 mg of Rilpivirine Hydrochloride was transferred in a clean and dry volumetric flask of 10 ml to which 0.1N HCL was added and made up to the mark and kept for 24 hours at room temp. From that 0.5 ml was taken in a volumetric flask of 10 ml and made up to the mark with diluents, then injected into the HPLC system and compared with the control sample.

Alkaline hydrolysis:

An accurately weighed amount of 10 mg of Rilpivirine Hydrochloride was transferred in a clean and dry volumetric flask of 10 ml to which 0.1 N NaOH added and made up to the mark and kept for 24 hours at room temp. From that 0.5 ml was taken in a volumetric flask of 10 ml and made up to the mark with diluents, then injected into the HPLC system and compared with the control sample.

Thermal degradation:

An accurately weighed amount of 10 mg of Rilpivirine Hydrochloride was transferred in a clean and dry volumetric flask of 10 ml, made up to the mark with diluent and was maintained at 70^{o} C in an oven for 48 hours. From that 0.5 ml was taken in a volumetric flask of 10 ml and made up to the mark with diluents and injected into the HPLC system and compared with the control sample.

Oxidation with (6%) H_2O_2:

An accurately weighed amount of 10 mg of Rilpivirine Hydrochloride was transferred in a clean and dry volumetric flask of 10 ml, To which 6% H_2O_2 added and made up to the mark and kept for 24hours at room temp. From that 0.5 ml was taken in 10 ml of volumetric flask and made up to the mark with diluents, then injected into the HPLC system and compared with the control sample.

Photodegradation:

An accurately weighed amount of 20 mg of Rilpivirine Hydrochloride was transferred in a clean and dry petri dish and was kept in direct sunlight for 24 hours without interruption. Accurately weighed 10 mg of exposed drug was transferred to a clean and dry volumetric flask of 10 ml. From that 0.5 ml was taken in 10 ml of volumetric flask and made up to the mark with diluent and injected into the HPLC system and compared with the control sample.

RESULT AND DISCUSSION

Selection and Optimization of Detection Wavelength:

The λ max of Rilpivirine Hydrochloride is 305 nm.

Statistical data analysis (DOE)

Table 06 shows Summary of factor and their level selected for 3^3 Box Behnken full factorial design.

Table 06: Summary of factors

Factor	Name	Units	Type	Minimum	Maximum	Mean
A	Methanol	%	Numeric	75.00	85.00	80.00
B	Flow Rate	ml/min	Numeric	0.8000	1.20	1.0000
C	pH	unit	Numeric	4.80	5.20	5.00

Analysis of variance for the Retention Time response as the dependent variable:

A) Results for the retention time of DOE:

ANOVA for response surface linear model

The analysis of variance (ANOVA) was performed to identify significant and insignificant factors. Table 07 represents results of ANOVA for the retention time of DOE:

Table 07: ANOVA table for Retention Time

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	27.22	3	9.07	21.63	< 0.0001	
A-Methanol	18.95	1	18.95	45.18	< 0.0001	
B-Flow Rate	8.26	1	8.26	19.7	0.0007	significant
C-pH	0.0084	1	0.0084	0.02	0.8897	
Residual	5.45	13	0.4194			
Lack of Fit	4.92	9	0.5464	4.09	0.094	not significant
Pure Error	0.5341	4	0.1335			
Cor Total	32.67	16				

Sum of squares is **Type III - Partial**

The **Model F-value** of 21.63 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The **Lack of Fit F-value** of 4.09 implies there is a 9.40% chance that a Lack of Fit F-value this large could occur due to noise. Lack of fit is bad -- we want the model to fit. This relatively low probability (<10%) is troubling.

B) Model assessment for the retention time response as the dependent variable:

After entering the data in Design-Expert software, fit summary applied to data after which the "quadratic model" was suggested by the software. According to this model following polynomial equation was obtained. The polynomial equation in coded terms (Table 08),

Table 08: Final Equation in Terms of Coded Factors:

Retention Time =	$+6.16 - 1.54 * A - 1.02 * B - 0.0324 * C$

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

C) Graphical Presentation: Retention Time:

Figure 02, 03 , 04 shows different contour plots for Retention Time.

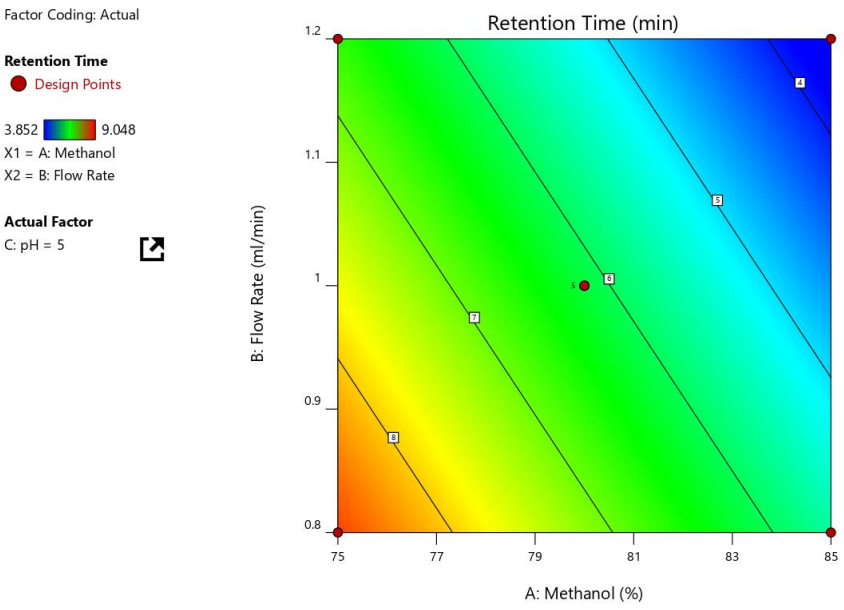


Figure 02: Contour plot for Retention Time (min) against Flow Rate and Mobile Phase-Methanol (AB)

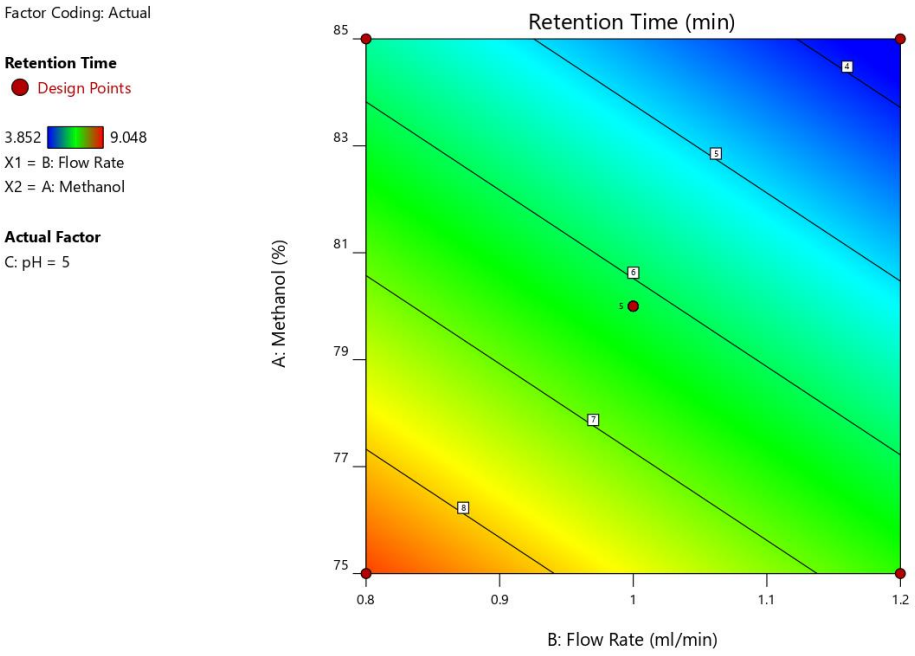


Figure 03: Contour plot for Retention Time (min) against Mobile Phase-Methanol and Flow Rate (BA)

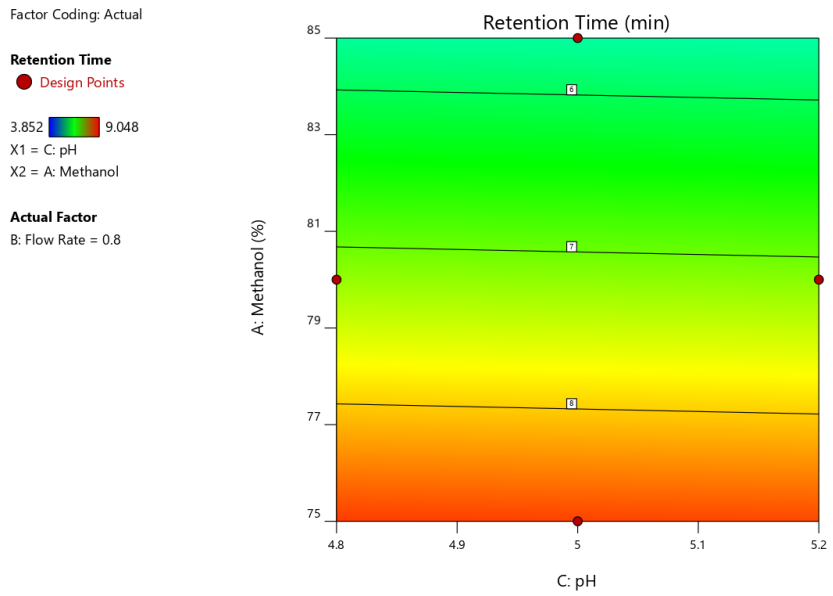


Figure 04: Contour plot for Retention Time (min) against Mobile Phase-Methanol and pH (CA)

Analysis of variance for the USP Plate Count response as the dependent variable:

A) Results for the USP Plate Count of DOE:

ANOVA for response surface linear model

The analysis of variance (ANOVA) was performed to identify significant and insignificant factors. Table 09 represents results of ANOVA for the USP Plate Count of DOE:

Table 09: ANOVA table for USP Plate Count

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2.47E+07	3	8.23E+06	3.56	0.0447	significant
A-Methanol	1.60E+06	1	1.60E+06	0.6923	0.4204	
B-Flow Rate	1.88E+07	1	1.88E+07	8.12	0.0137	
C-pH	4.30E+06	1	4.30E+06	1.86	0.1961	
Residual	3.01E+07	13	2.32E+06			
Lack of Fit	3.00E+07	9	3.34E+06	150	0.0001	significant
Pure Error	88935.94	4	22233.99			
Cor Total	5.48E+07	16				

Sum of squares is **Type III - Partial**

The **Model F-value** of 3.56 implies the model is significant. There is only a 4.47% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case, B is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The **Lack of Fit F-value** of 150.00 implies the Lack of Fit is significant. There is only a 0.01% chance that a Lack of Fit F-value this large could occur due to noise. A significant lack of fit is bad -we want the model to fit.

B) Model assessment for the retention time response as the dependent variable:

After entering the data in Design-Expert software, fit summary applied to data after which the "quadratic model" was suggested by the software. According to this model following polynomial equation was obtained. The polynomial equation in coded terms (Table No. 10):

Table 10: Final Equation in Terms of Coded Factors:

USP Plate Count =	$+10173.71 + 447.66 * A - 1532.88 * B + 733.16 * C$

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

C) Graphical Presentation: USP Plate Count

Figure 05, 06, 07 shows different contour plots for USP Plate Count.

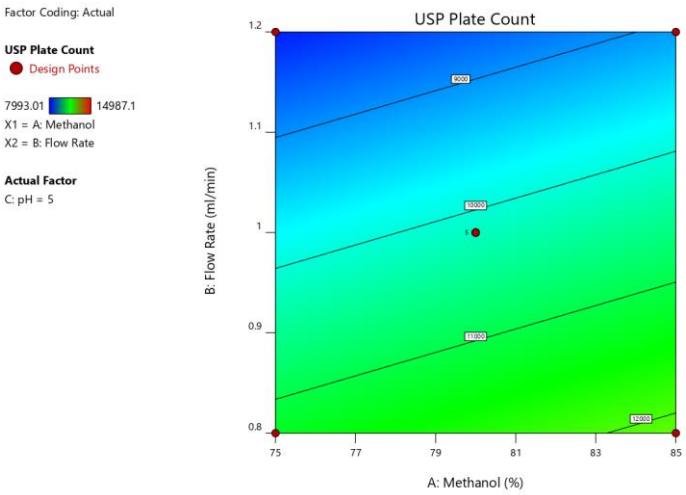


Figure 05: Contour plot for USP Plate Count against Flow Rate and Mobile Phase-Methanol (AB)

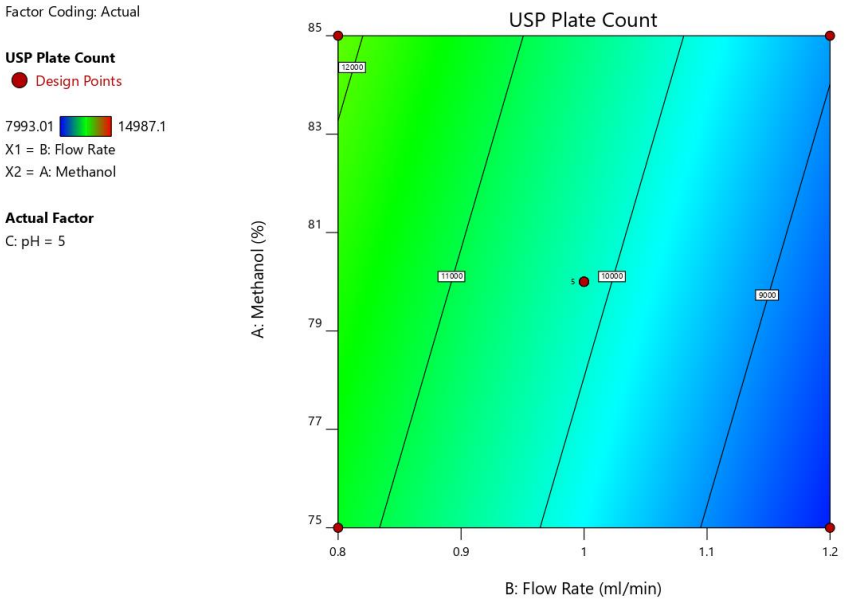


Figure 06: Contour plot for USP Plate Count against Mobile Phase-Methanol and Flow Rate (BA)

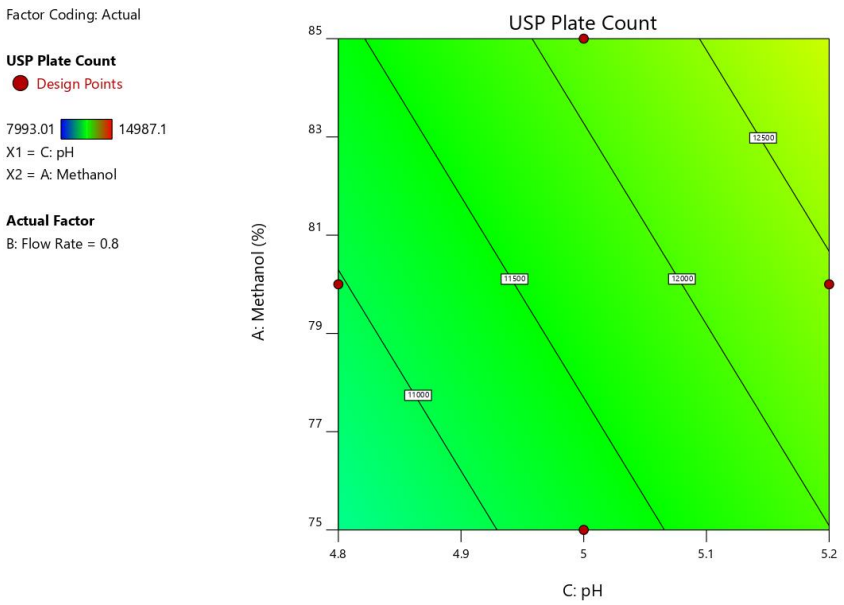


Figure 07: Contour plot for USP Plate Count against Mobile Phase-Methanol and pH (CA)

Analysis of variance for the USP Tailing:

A) Response as a dependent variable:

ANOVA for response surface linear model

The analysis of variance (ANOVA) was performed to identify significant and insignificant factors. Table 11 represents results of ANOVA for the USP Tailing Factor of DOE:

Table 11: ANOVA table for USP Tailing Factor

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0029	9	0.0003	4.49	0.0301	significant
A-Methanol	0	1	0	0	1	
B-Flow Rate	0.0003	1	0.0003	4.33	0.0759	
C-pH	0.0003	1	0.0003	4.33	0.0759	
AB	0.0001	1	0.0001	1.39	0.2775	
AC	0.0009	1	0.0009	12.48	0.0096	
BC	0	1	0	0.3465	0.5746	
A^2	0.0004	1	0.0004	5.55	0.0507	
B^2	0.0003	1	0.0003	3.51	0.1033	
C^2	0.0006	1	0.0006	8.76	0.0211	
Residual	0.0005	7	0.0001			
Lack of Fit	0.0004	3	0.0001	7.08	0.0445	Significant
Pure Error	0.0001	4	0			
Cor Total	0.0034	16				

Sum of squares is **Type III - Partial**

The **Model F-value** of 4.49 implies the model is significant. There is only a 3.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case AC, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The **Lack of Fit F-value** of 7.08 implies the Lack of Fit is significant. There is only a 4.45% chance that a Lack of Fit F-value this large could occur due to noise. A significant lack of fit is bad -we want the model to fit.

B) Model assessment for the retention time response as the dependent variable:

After entering the data in Design-Expert software, fit summary applied to data after which the "quadratic model" was suggested by the software. According to this model following polynomial equation was obtained. The polynomial equation in coded terms (Table 12),

Table 12: Final Equation in Terms of Coded Factors:

USP Tailing =	$+1.07 + 0.0000 * A - 0.0062 * B + 0.0062 * C - 0.0050 * AB - 0.0150 * AC + 0.0025 * BC - 0.0098 * A^2 + 0.0077 * B^2 - 0.0122 * C^2$

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

C) Graphical Presentation: USP Tailing

Figure 08, 09, 10 shows different contour plots for USP Tailing.

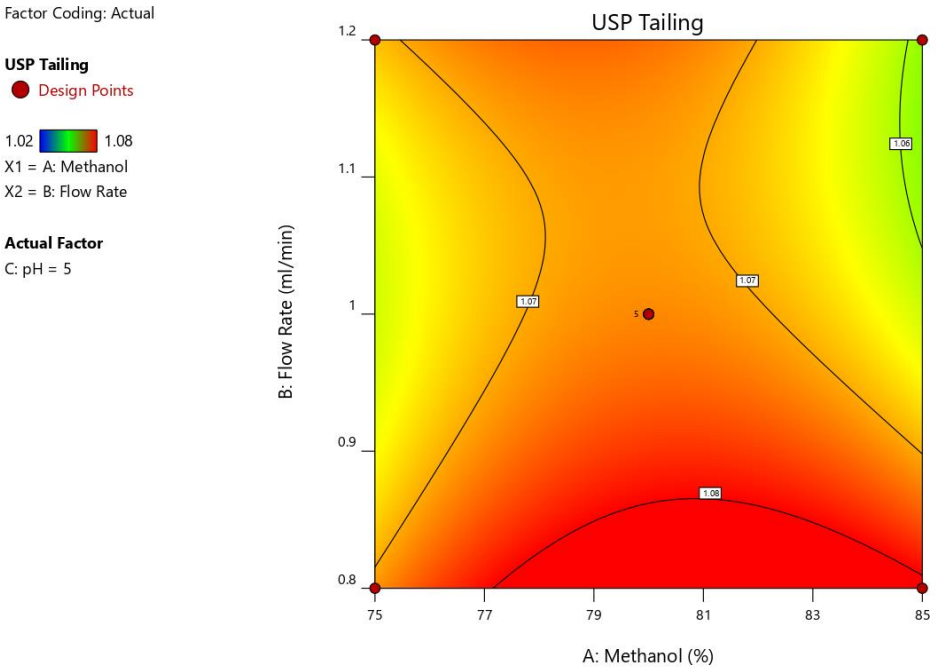


Figure 08: Contour plot for USP Tailing against Flow Rate and Mobile Phase-Methanol (AB)

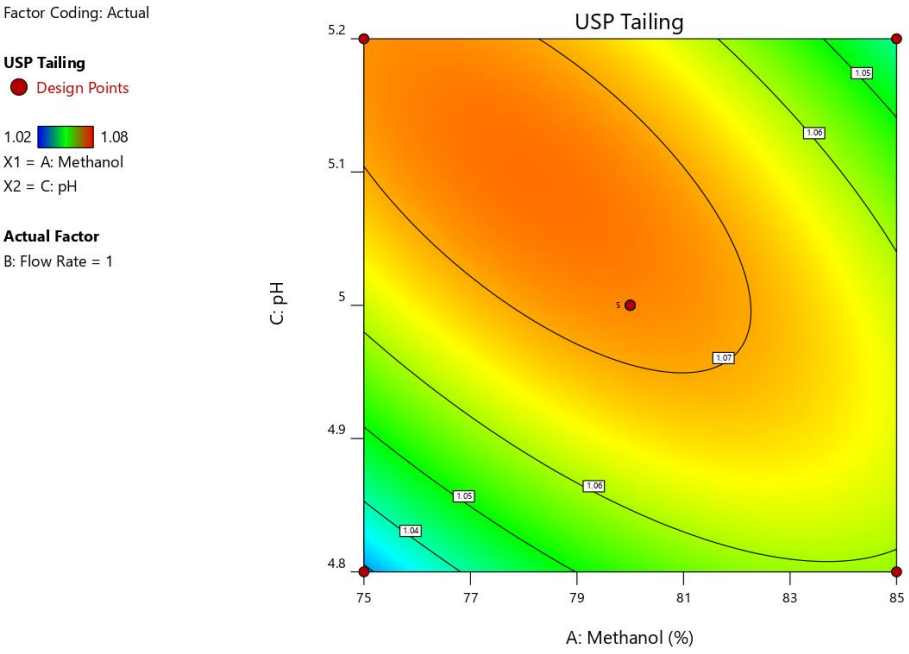


Figure 09: Contour plot for USP Tailing against pH and Mobile Phase-Methanol (AC)

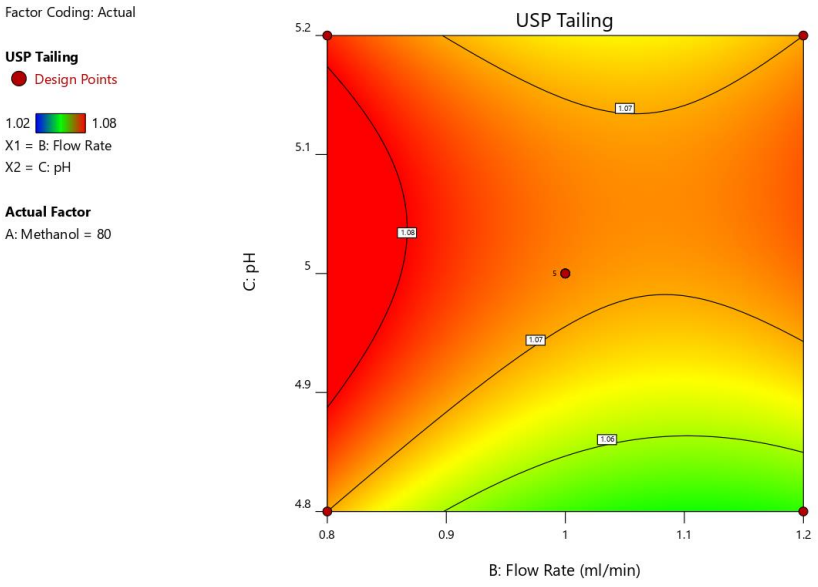


Figure 10: Contour plot for USP Tailing against pH and Flow Rate (BC)

Developed Method Operable Design Region:

Design Space for study DOE:

The graphical optimization done by with the help of Design-Expert software provided the base to define the design space as shown in following Figure 11, 12, 13.

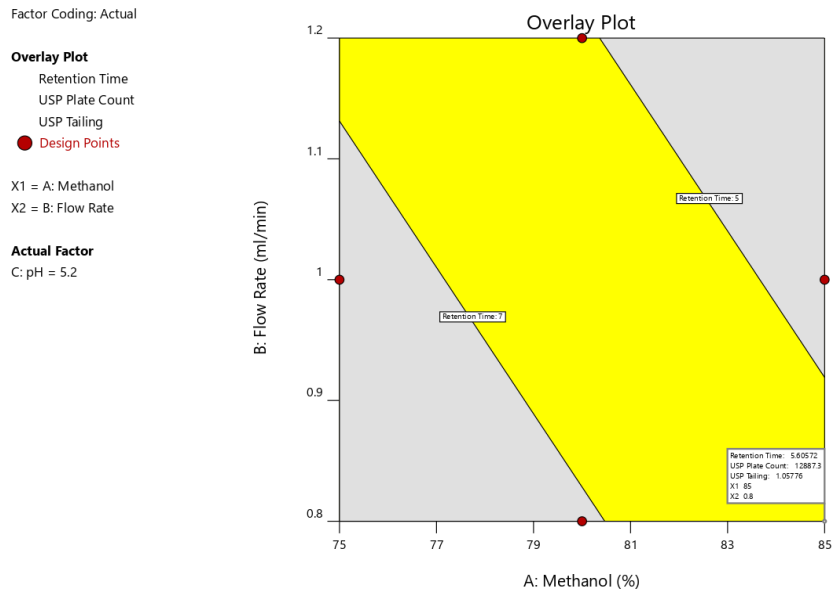


Figure 11: Overlay plot for Flow Rate and Mobile Phase-Methanol (AB)

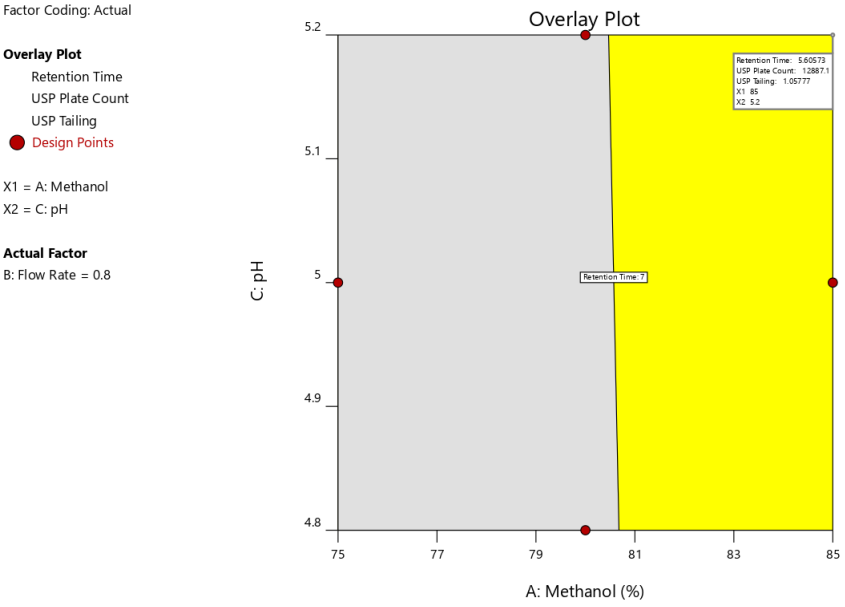


Figure 12: Overlay plot for pH and Mobile Phase-Methanol (AC)

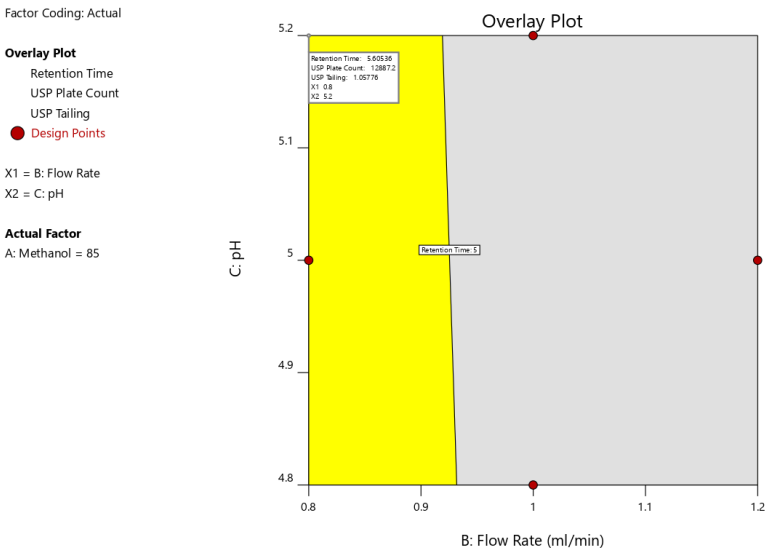


Figure 13: Overlay plot for pH and Flow Rate (BC)

This plot elaborates that the optimized values of both independent variables in the required target range of Retention Time, USP Plate Count and USP Tailing lie within the yellow region which is the useful optimum region where the design space can be determined whereas the grey colored region is restricted to achieve the target response value of a dependent variable.

Optimized Method gave by the software:

(Injection Volume: 10 µL)

Table 13 represents the final optimised conditions.

Table 13: Optimized Method

Flow Rate	pH	Mobile Phase Composition (mL)
0.8 ml/min	5.2	Methanol: Water (85:15)

System Suitability:

Table 14: System suitability test for Rilpivirine HCl

Rilpivirine HCl				
Sample Name (50ppm)	**Retention Time (min)**	**Area**	**USP Plate count**	**Tailing factor**
Standard 1	5.06	5241174	9126	1.07
Standard 2	5.31	5277168	9070	1.08
Standard 3	5.31	5247607	8976	1.07
Standard 4	5.29	5134424	8857	1.07
Standard 5	5.23	5110761	9199	1.07
	AVERAGE	5202227	-	-
	SD	74423.86	-	-
	%RSD	1.43	-	-

Validation of the Developed Method

Chromatographic conditions

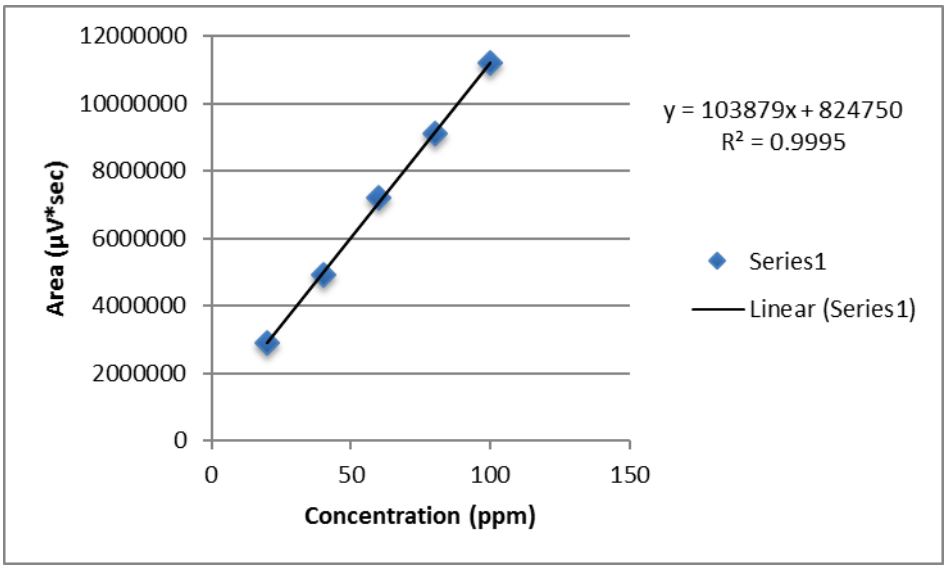
The following table 15 shows chromatographic conditions were established by trial and error and were kept constant throughout the experimentation.

Table 15: Optimized Conditions for Validation

HPLC	Waters
Detector and pump no.	2489 UV And 1525 binary pump
Software	BREEZE 2
Column	4.6 x 250 mm in length
Particle size packing	5 µm
Stationary phase	C_{18} (CHEMSIL ODS)
Mobile phase	Methanol: Water (85:15) ml
Detection Wavelength	305 nm
Flow Rate	0.8 ml/min
Temperature	Ambient
Sample size	10 µl

i. Linearity:

The graph 01 shows plot for linearity and Table 16 represents results for lineartity



Graph 01: Linearity Graph of Rilpivirine HCl

Table 16: Result and Statistical Data for Linearity

Conc (ppm)	Peak Area I	Peak Area II	Peak Area III	Mean Area
20	2947292	2839364	2898563	2895073
40	4968585	4846883	4931130	4915533
60	6797807	6411167	8354429	7187801
80	8157898	9576720	9555193	9096604
100	11343747	10508942	11724621	11192437
Equation			y = 103879x + 824750	
Slope			103879	
Intercept			824750	
Correlation Coefficient (R^2)			0.999	

Accuracy:

It was done by a recovery study. Sample solutions were prepared by spiking at about 50 %, 100 %, and 150 % of the specification limit to Placebo and analyzed by the proposed HPLC method. The results are shown in table 17.

Preparation of recovery stock solution: 25 mg of Rilpivirine Hydrochloride working standard was weighed accurately and transferred into 25 ml volumetric flask, methanol was added and sonicated to dissolve and finally the volume was made with diluents and mixed. The working standard concentration is 1000 µg/ml. Remove 1ml and dilute up to 10ml with the mobile phase to give 100 µg/ml solution.

Table 17: Result and statistical data of Accuracy

Sr. No.	Conc. Level	Conc. (µg/mL) Sample solution	Conc. (µg/mL) for stock solution	Area (VIN)	MEAN AREA ± SD	%RSD	AREA OF STD	% RSD
1	50%	50	25	5985141	5573359 ± 11482.7	0.19	6018000	99.25
		50	25	5972736				
		50	25	5962201				
2	100%	50	50	11147639	11171987 ± 58527.72	0.52	11212650	99.63
		50	50	11238758				
		50	50	11129563				
3	150%	50	75	16274778	16313616 ± 39644.75	0.24	16406600	99.43
		50	75	16312048				
		50	75	16354021				

Precision:

Preparation of standard solution:

Weigh accurately about 25 mg of Rilpivirine Hydrochloride and transferred to 25ml Methanol (Sonicate it to dissolve completely) to give 1000 ppm stock solution. From that stock, the solution takes 0.5 ml and dilute to 10ml with the mobile phase get 50 ppm solution. Table 18 shows results for intraday precision.

Table 18: Result and Statistical data For Intraday Precision

I) INTRADAY PRECISION

Sr. No.	Conc. (ppm)	Area	Mean	SD	% RSD
1	50	5049224	5048752	28231.07	**0.55**
2	50	5019312			
3	50	5101309			
4	50	5031275			
5	50	5041717			
6	50	5049677			

II) INTERDAY PRECISION

Table 19 shows results for interday precision

Table 19: Result and Statistical data For Interday Precision

Sr. No.	Conc. (ppm)	Area	Mean	SD	% RSD
1	50	5049874	5024572	58058.52	**1.15**
2	50	4986183			
3	50	5028256			
4	50	4931130			
5	50	5092654			
6	50	5060384			

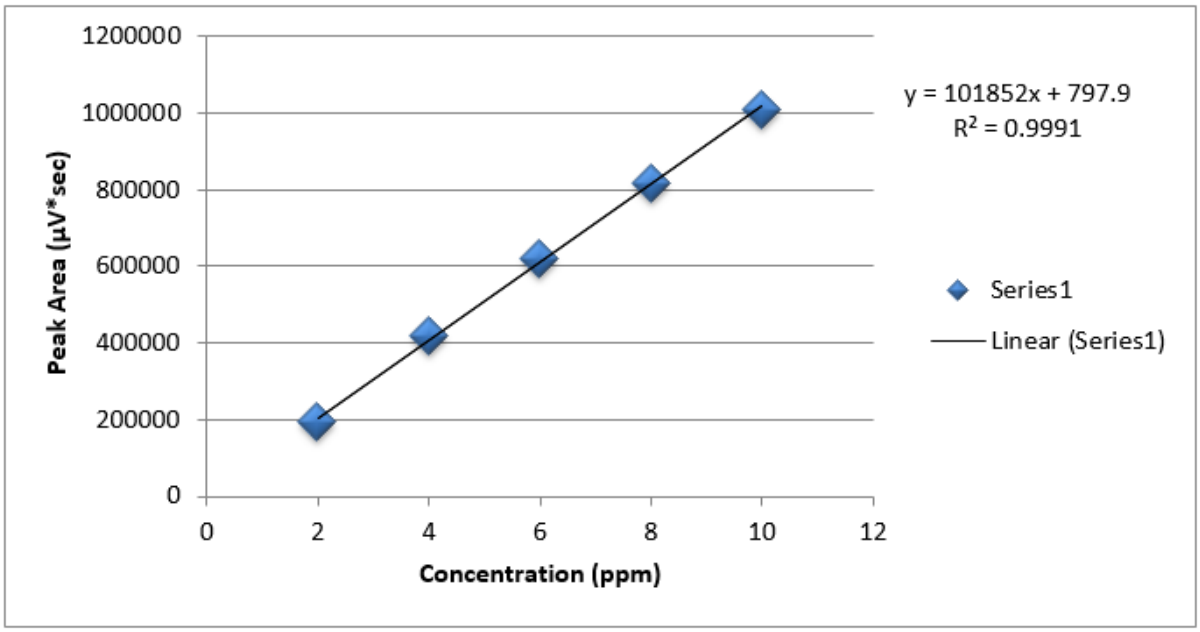
Limit of Detection (LOD):

It may be calculated based on the standard deviation (SD) of the response and slope of the calibration curve(S).

Graph 02 shows the plot of LOD and LOQ and Table 20 represents result for LOD and LOQ.

Table 20: Result and statistical data of LOD & LOQ of Rilpivirine HCl

Rilpivirine HCl					
Sr. No.	**Concentration (µg/ml)**	**RT (min)**	**Area (µV*sec)**	**USP Plate Count**	**USP Tailing**
1	2	5.256	190172	10463	1.09
2	4	5.200	419467	9453	1.08
3	6	5.231	619229	9414	1.09
4	8	5.271	816586	10144	1.08
5	10	5.178	1012119	8940	1.08
Correlation Coefficient			0.999		
Slope			101852		
SD			322234.7		
LOD (µg/ml)			**1.04**		
LOQ (µg/ml)			**3.16**		



Graph 02: Calibration Curve for LOD & LOQ of Rilpivirine HCl

$$\mathbf{LOD = 3.3\ X\ \frac{(SD)}{S}}$$

Where, **SD=** Standard deviation

S= Slope

Calculation of Rilpivirine Hydrochloride:

$$\text{LOD} = \mathbf{3.3\ X\ \frac{(32223.7)}{101852}}$$

$$= 1.04$$

$$\text{LOD} = 1.04\ \mu g/\ ml$$

LOD of Rilpivirine Hydrochloride was found to be **1.04 µg/ ml.**

Limit of Quantitation (LOQ):

It may be calculated based on the standard deviation (SD) of the response and slope of the curve(S).

$$\mathbf{LOQ = 10\ X\ \frac{(SD)}{S}}$$

Where, **SD =** Standard deviation

S= Slope

Calculation of Rilpivirine Hydrochloride:

$$\text{LOQ} = \mathbf{10\ X\ \frac{(32223.7)}{101852}}$$

$$= 3.16$$

$$\text{LOQ} = 3.16\ \mu g/ml$$

LOQ of Rilpivirine Hydrochloride was found to be **3.16µg/ml**

Forced degradation study:

1. Control sample

Figure 14 shows chromatogram of control sample and Table 22 represents chromatogram data for control sample.

2. Acid degradation

Figure 15 shows chromatogram of acidic sample and Table 23 represents chromatogram data for acidic sample.

3. Base degradation

Figure 16 shows chromatogram of basic sample and Table 24 represents chromatogram data for basic sample.

4. Peroxide degradation

Figure 17 shows chromatogram of oxidation sample and Table 25 represents chromatogram data for oxidation sample.

5. Thermal degradation

Figure 18 shows chromatogram of thermal sample and Table 26 represents chromatogram data for thermal sample.

6. Photolytic degradation

Figure 19 shows chromatogram of photolytic sample and Table 27 represents chromatogram data for photolytic sample.

Following table 21 shows results for stability study:

Table 21: Result and Statistical data for Stability study of Rilpivirine HCl

Sr. No.	Stress condition	Degradation time	Peak area	% of Degradation	% Active drug remaining
1	Control	-	5163031	-	-
2	Acid (1N HCl)	24 hrs.	4763117	7.75	92.25
3	Base (1N NaOH)	24 hrs	5048132	2.23	97.77
4	Oxidation(6%H_2O_2)	24 hrs	4590208	11.10	88.90
5	Thermal (70 ^oC)	24 hrs	4354691	15.66	84.34
6	Photolytic	24hrs	4995713	3.25	96.75

Control sample:

- Wavelength: 305 nm
- Mobile phase: Methanol: Water (85:15) pH 5.2
- Sample volume: 10µl
- Flow rate: 0.8ml/min

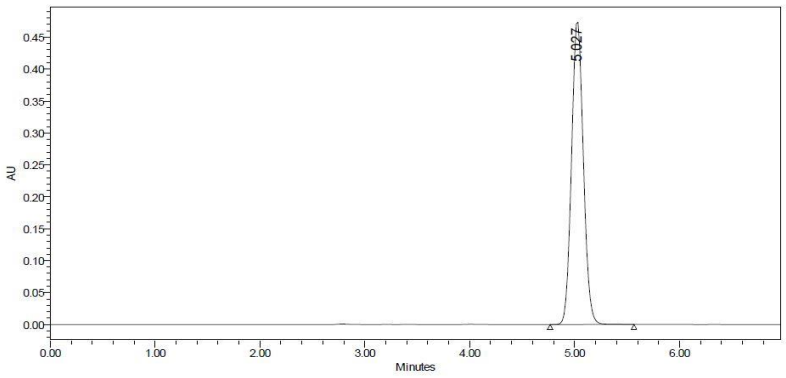


Figure 14: Chromatogram of Control Sample

Table 22: Chromatogram Data for Control Sample

Sr. No.	Compound	RT (min)	Area (µV*sec)	USP Plate Count	USP Tailing
1	Rilpivirine HCl	5.0	5163031	9548	1.07

Acidic degradation:

- Wavelength: 305 nm
- Mobile phase: Methanol: Water (85:15) pH 5.2
- Sample volume : 10µl
- Flow rate: 0.8ml/min

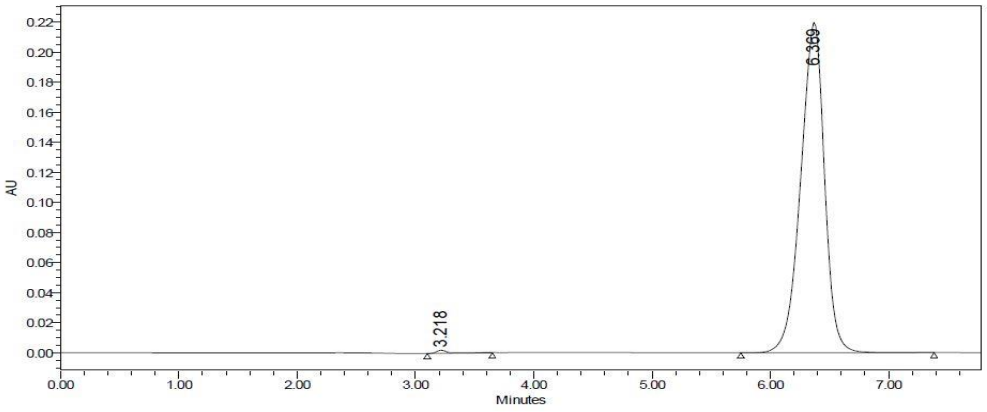


Figure 15: Chromatogram of Acidic Sample

Table 23: Chromatogram Data for Acidic Sample

Sr. No.	Compound	RT (min)	Area (µV*sec)	USP Plate Count	USP Tailing
2	Rilpivirine HCl	6.3	4763117	4767	0.9

Basic degradation:

- Wavelength: 305 nm
- Mobile phase: Methanol: Water (85:15) pH 5.2
- Sample volume : 10µl
- Flow rate: 0.8ml/min

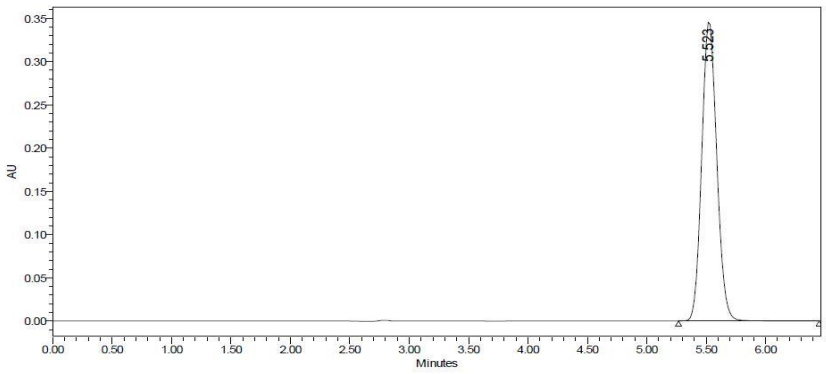


Figure 16: Chromatogram of Basic Sample

Table 24: Chromatogram Data for Basic Sample

Sr. No.	Compound	RT (min)	Area (µV*sec)	USP Plate Count	USP Tailing
3	Rilpivirine HCl	5.5	5048132	8896	1.09

Oxidation degradation:

- Wavelength: 305 nm
- Mobile phase: Methanol: Water (85:15) pH 5.2
- Sample volume : 10µl
- Flow rate: 0.8ml/min

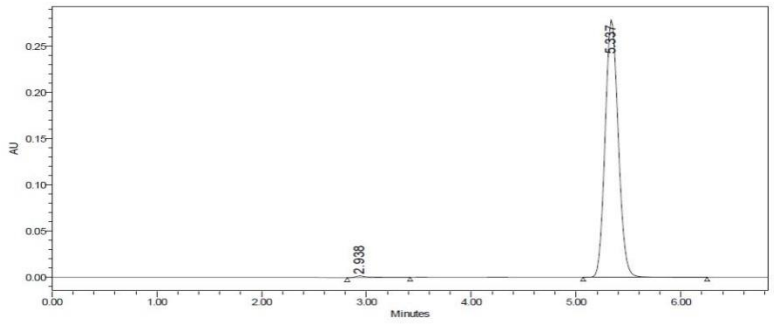


Figure 17: Chromatogram of Oxidation Sample

Table 25: Chromatogram Data for Oxidation Sample

Sr. No.	Compound	RT (min)	Area (µV*sec)	USP Plate Count	USP Tailing
4	Rilpivirine HCl	5.3	2410202	8652	1.1

Thermal degradation:

- Wavelength: 305 nm
- Mobile phase: Methanol: Water (85:15) pH 5.2
- Sample volume: 10µl
- Flow rate: 0.8ml/min

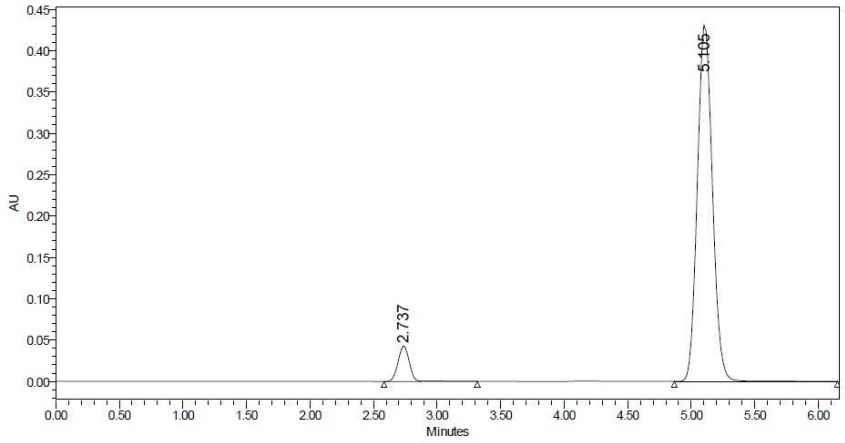


Figure No. 18: Chromatogram of Thermal Sample

Table No. 26: Chromatogram Data for Thermal Sample

Sr. No.	Compound	RT (min)	Area (µV*sec)	USP Plate Count	USP Tailing
5	Rilpivirine HCl	5.105	4354691	8763	1.1

Photolytic Degradation:

- Wavelength: 305 nm
- Mobile phase: Methanol: Water (85:15) pH 5.2

- Sample volume: 10µl
- Flow rate: 0.8ml/min

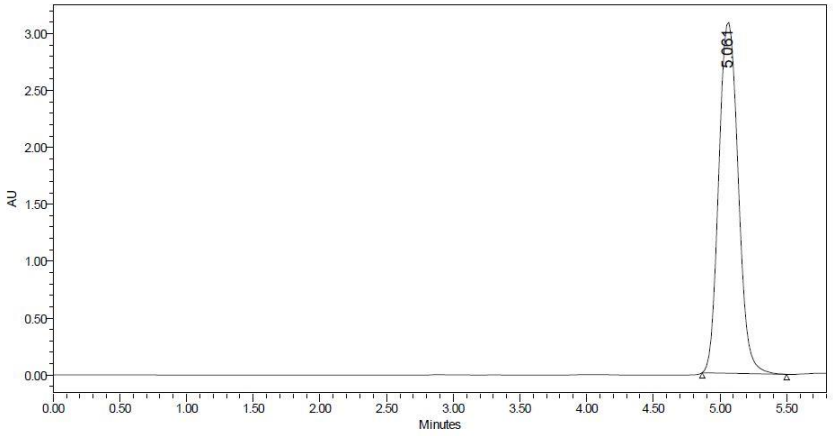


Figure 19: Chromatogram of Photolytic Sample

Table 27: Chromatogram Data for Photolytic Sample

Sr. No.	Compound	RT (min)	Area (µV*sec)	USP Plate Count	USP Tailing
6	Rilpivirine HCl	5.0	4995713	6037	1.1

CONCLUSION

Quality by Design approach has been successfully used for Development of RP-HPLC Method for estimation of Rilpivirine HCl. The developed method employed mobile phase Methanol: Water (85:15) (pH 5.2) pH and flow rate 0.8 ml/min, which was optimized with the help of design expert software. Linearity of the developed method was confirmed over concentration range of 20-100 µg/mL for Rilpivirine HCl with correlation coefficient of 0.999. The percentage RSD for precision and accuracy of the method was found to be less than 2%. Stability indicating studies (forced degradation) of Rilpivirine HCl was carried out at for 24 hours under various conditions like acidic, alkali, oxidation, thermal, photolytic and degradation of Rilpivirine HCl was found to be 7.75%, 2.23%, 11.10%, 15.66%, 3.25% respectively. Rilpivirine HCl was found to degrade under various conditions. Moreover, the lower solvent consumption along with the short analytical run time of 10 min leads to a cost effective and environmentally friendly chromatographic procedure. Thus, the proposed

methodology is rapid, selective, requires a simple sample preparation procedure, and represents a good procedure for Rilpivirine HCl.

ACKNOWLEDGMENT

The completion of this research is not only the fulfillment of our dreams but also the dreams of our family who have taken lots of pain for us in the completion of our higher studies. We take this privilege and pleasure to acknowledge the contributions of many individuals who have been inspirational and supportive throughout our work undertaken and endowed us with the most precious knowledge to see success in our endeavor. We sincerely thank to our esteemed guide and colleagues for helping us in project work and M. V. P Samaj's College of Pharmacy, Nashik for providing us a platform for research work.

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