

A QBD APPROACH FOR HPLC METHOD DEVELOPMENT, VALIDATION AND FORCED DEGRADATION STUDIES OF RILPIVIRINE

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Abstract: The present study set out to create and validate a reliable and precise HPLC method for estimating Rilpivirine, employing a systematic Quality by Design (QbD) approach. We optimized key method parameters, including flow rate and mobile phase composition, through risk assessment and Central Composite Design (CCD). The final chromatographic conditions featured a C18 column, a mobile phase made up of phosphate buffer (pH 3.6) and acetonitrile in a 40:60 v/v ratio, a flow rate of 1.0 mL/min, UV detection at 298 nm. Under these settings, Rilpivirine exhibited a retention time of 3.3 minutes with excellent peak symmetry. We validated the method in accordance with ICH Q2(R2) guidelines, achieving remarkable linearity across a concentration range of 10–50 ppm, with an R^2 value of 0.9973. Accuracy tests revealed recoveries ranging from 98.88% to 108.15%, while precision assessments yielded %RSD values below 1%, confirming the method's reliability. The limits of detection (LOD) and quantification (LOQ) were determined to be 2.97 µg/mL and 9 µg/mL, respectively. Forced degradation studies conducted under acidic, alkaline, oxidative, thermal, and photolytic conditions indicated degradations of 13.04%, 19.87%, 18.2%, 19.7%, and 15.2%, respectively. These findings validated that the developed method could effectively separate Rilpivirine from its degradation products, highlighting its stability-indicating capabilities. The HPLC method proved to be precise, cost-effective, and well-suited for routine quality control and stability assessments of Rilpivirine formulations.

Keywords: HPLC, Method development, Method validation, Forced degradation, ICH guidelines, Design of Experiment.

Abbreviations and notation: QbD, Quality by Design; CCD, Central composite design; ICH, International Council for Harmonization; LOD, Limit of Detection; LOQ, Limit of Quantification; CMP, Critical Method Parameters; CQA, Critical Quality Attributes; ACN, Acetonitrile; RSD, Relative Standard Deviation.

INTRODUCTION

In the world of pharmaceuticals, creating strong, dependable, and reproducible analytical methods is crucial for ensuring that drug products are of high quality, safe, and effective[1]. One of the go-to techniques for analyzing pharmaceutical compounds is High-Performance Liquid Chromatography (HPLC), known for its impressive sensitivity, accuracy, and precision in both quantitative and qualitative analysis[2]. Quality by Design (QbD) has become increasingly important in developing analytical methods. QbD involves pinpointing critical method parameters (CMPs) and critical quality attributes (CQAs), along with conducting risk assessments and designing experiments (DoE) to ensure the method is robust and performs consistently[3]. Rilpivirine, a non-nucleoside reverse transcriptase inhibitor (NNRTI), is used to treat Human Immunodeficiency Virus (HIV) infection[4]. Given its therapeutic significance, it's vital to create a stability-indicating analytical method that can accurately measure the drug even in the presence of its degradation products.

Forced degradation studies are essential for confirming the stability-indicating nature of an analytical method. These studies expose the drug substance to various stress conditions like acidic, basic, oxidative, thermal, and photolytic environments to produce potential degradation products[5]. The insights gained from these studies help us understand degradation pathways, intrinsic stability, and the best storage conditions according to ICH Q1A(R2), Q1B[6]. This study aims to develop and validate a robust HPLC method for estimating Rilpivirine using a QbD approach. Additionally, the method will be assessed through forced degradation studies to showcase its specificity and stability-indicating capabilities, all in line with regulatory guidelines.

METHODS AND MATERIAL

CHEMICALS AND REAGENT:

Rilpivirine pure standard (99%) was received as a gift sample from Emcure Pharmaceuticals, Hinjewadi. In addition, acetonitrile, water, and orthophosphoric acid (OPA), methanol (HPLC-grade) were used.

INSTRUMENTATION:

The study was conducted with a Jasco HPLC instrument with an UV detector. Additional equipment included an analytical balance (Mettler Toledo) and an ultrasonicator. The chromatographic separation was performed using a C 18 (I.D. = 250× 4.6 mm) column.

Preparation of the Diluent: A 60:40 (v/v) ACN: Phosphate buffer solution mixture was prepared based on the solubility of the drug.

Preparation of the Mobile Phase: The mobile phase was prepared by mixing buffer (KH₂PO₄) and Acetonitrile in a 40:60 (v/v) ratio based on the optimized method conditions.

Preparation of the Standard Stock Solution: A precisely weighed 10 mg Rilpivirine was transferred to a 10 mL volumetric flask. Subsequently, 10 mL of the methanol was introduced and agitated for 10 min in a sonicator and mixture was filtered through a 0.45 µm filter, and 1mL of the filtrate was diluted to 10 mL with the diluent, yielding a final concentration of 100 µg/mL Rilpivirine.

Method Development and Optimization Using the QbD Approach:

Identification of the Analytical Target Profile, Critical Method Attributes, and Risk Assessment: ATP was established through a literature factors influencing Rilpivirine quantification via HPLC. The chosen critical method parameters were related to different parameters of HPLC (i.e., flow rate, and buffer ratio)[7].

Method Development and Optimization Using Central Composite Design: The method optimization employed CCD, which is commonly used for second-order models.

Two key factors, buffer concentration, flow rate, were optimized across three levels (high, mid, and low) within specific ranges: phosphate buffer and a 1mL/min flow rate. Contour and 3D surface plots were generated to illustrate the impact of each on critical quality attributes (CQAs) such as peak area, retention time, and asymmetry. A desirability function was employed to predict these CQAs under the optimized conditions[8]. The detailed experimental design for Rilpivirine injection is presented in Table 1

Risk Assessment and Control Strategy: To assess its efficacy and durability, the optimized approach was examined for critical material attributes. Consequently, the method's performance and parameters were assessed for robustness under a number of specifically selected conditions. Minor adjustments to crucial parameters, including flow rate and percentage of the buffer phase, were made to assess the robustness of the method[9].

Chromatographic Conditions: Rilpivirine was tested in pharmaceutical formulations and degradation investigations using a Sun Internationals C18column (5 µm particle size, L × I.D. = 250× 4.6 mm). The mobile phase, consisting of the buffer (KH₂PO₄ PH 3.6) and Acetonitrile in a ratio of 40:60 (v/v), was used at a flow rate of 1mL/min. The analysis was performed utilizing a UV detector at 298 nm, with 10.0 µL injections of sample solutions.

Method Validation: The HPLC method for Rilpivirine injection was verified for linearity, precision, accuracy, and limits of quantification and detection, as per the ICH Q2 (R2) guidelines[10].

Linearity: Rilpivirine (10 mg) was precisely weighed and then placed in a 10 mL volumetric flask. After adding 10 mL of methanol and sonicating for 20 min, this create a standard stock solution (1000 ppm Rilpivirine). The linearity of the procedure was determined by examining successive dilutions of the Rilpivirine stock solution between 10 and 50 ppm.

Accuracy: Accuracy was assessed by analyzing Rilpivirine solutions spiked with 80%, 100%, and 120% standard Rilpivirine. Recovery and % RSD were calculated to verify the accuracy of the data.

Precision: Precision was evaluated through Intraday and Interday precisions, as per ICH guidelines, using three replicates of three different dilutions for intraday and Interday assessments.

Limit of Detection: The LOD was calculated using the formula $LOD = 3.3 \times \sigma/s$ (where σ is the standard deviation and s is the slope of the calibration curve).

Limit of Quantification: The LOQ was determined similarly using Rilpivirine solutions. It was calculated using the formula $LOQ = 10 \times \sigma/s$.

FORCED DEGRADATION STUDIES

Acid Degradation Analysis: A mixture of 2mL of the Rilpivirine stock solution and 2mL of 0.1N hydrochloric acid was heated at 60 °C for 3 hrs. The resulting mixture was then diluted to a 20 ppm solution. A 10 µL sample was injected into the system, and the chromatogram was observed to evaluate the stability of the sample.

Alkali Degradation Analysis: A combination of 2mL of the Rilpivirine stock solution and 2 mL of 0.1N sodium hydroxide solution was heated at 60 °C for 3 hrs. The resulting mixture was then diluted to a 20 ppm solution. A 10 µL sample was injected into the system, and a chromatogram was observed to evaluate the stability of the sample.

Oxidative Degradation Analysis: 2 mL of 20% hydrogen peroxide (H₂O₂) was mixed with 2 mL of a Rilpivirine stock solution for the oxidation experiments. To test the stability of the sample, the solution was diluted to 20 ppm, and 10 µL was injected into the HPLC system.

Thermal Degradation Analysis: The standard drug solution was exposed to 60°C for 6 hr in an oven to examine thermal degradation. The resulting solution was diluted to 20 ppm for HPLC analysis. A 10µL sample was injected into the system, and the chromatogram was analyzed to evaluate the stability of the sample.

Light Sensitivity Analysis: The photochemical stability of the drug was examined by exposing a 20 ppm solution to direct sunlight for 8 hrs. The resulting solution was diluted with the prepared diluent for the HPLC analysis. A 10 µL sample was introduced into the system, and the chromatogram was analyzed to assess the stability of the sample[11].

RESULTS AND DISCUSSION

Design of the Experiment (Optimized Method and Development): A factorial design was conducted with Design-Expert software 13.0(CCD) to observe the effect of two independent variables flow rate (FR,mL/min), organic ratio of the mobile phase (MP %) on three responses: retention period (RT), peak area (PA) and symmetry factor (SF). These parameters were optimized for the proposed method. Several characteristics within the design space were further optimized by using the CCD as shown in Table 2

Statistical Analysis: ANOVA was used to analyze the QbD data. The experimental data were analyzed using response surface methodology to evaluate the effect of formulation variables, namely flow rate (A) and buffer concentration (B), on retention time, peak area, and symmetry factor.

For retention time (Response 1), The Model F-value of 355.38 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, AB, B² are significant model terms. The Lack of Fit F-value of 612.04 implies the Lack of Fit is significant. The Predicted R² of 0.9748 is in reasonable agreement with the Adjusted R² of 0.9933; i.e. the difference is less than 0.2.

For peak area (Response 2), The Model F-value of 6.78 implies the model is significant. There is only a 1.38% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant.

For symmetry factor (Response 3), The Model F-value of 15.11 implies the model is significant. There is only a 0.12% 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, AB, B² are significant model terms. The Lack of Fit F-value of 7.04 implies the Lack of Fit is significant.

The optimized method parameters for HPLC analysis of Rilpivirine are presented in Table 3 and Figure 8.

Method Validation:

Linearity: The linearity of the method was demonstrated by using a correlation between the area response and sample concentration over a working concentration range. This ensures that the response is proportional to the concentration. The linear range for Rilpivirine was found to be 10 to 50 ppm. The regression equation of the calibration curve was $y = 112607x + 59924$ with a correlation coefficient of 0.9973. The graph was plotted with peak area vs concentration (Figure 9), indicating a linear response.

Accuracy: To assess the accuracy of the approach, Rilpivirine was added at three different concentration levels (80%, 100%, and 120%). The %mean recoveries were 98.88%, 108.15%, and 100.75%, with a % RSD of 0.62%. Results in Table 4 indicate that the recovery percentages ranged between 98% and 108%, indicating that the developed approach is precise and suitable for analysis of Rilpivirine. Table 4

Precision: Three replicative injections of three different solutions (30 ppm, 40 ppm, 50 ppm) were introduced into the HPLC column. The area response and % RSD of the Rilpivirine peak are shown in Table 5 and 6, indicating an appropriate degree of precision for the HPLC technique. The % RSD for intraday was 0.029%, 0.88% and 0.56% and for inter-day was 1.33%, 1.07% and 1.003%, respectively indicating an acceptable level of precision (as per the acceptance criteria, RSD must be less than 2%).

LOD and LOQ: Based on the standard deviation of the slope and intercept, the LOD and LOQ for Rilpivirine were determined to be 2.97 and 9 µg/mL, respectively (as per the acceptance criteria, LOD must be ≥ 3 and LOQ must be ≥ 10).

Forced Degradation Studies: The degradation of Rilpivirine under varied stress conditions was further studied by following ICH Q1A and Q1B guidelines. Different stress conditions, as outlined in the protocol, were applied by using standard solutions. The drug showed 13.04% degradation under acidic conditions (Figure 10), 19.87% degradation under alkali conditions (Figure 11), and 18.2% degradation under oxidation conditions (Figure 12). In thermal and photolytic conditions, the drug showed 19.7% and 15.2% degradation (Figures 13 and 14). Degradation of drug substances between 5% and 20% is acceptable for the validation of chromatographic assays. In Table 7, the data of Analysis of Forced degradation studies is represented.

CONCLUSION

The Quality by Design (QbD) approach has been used to develop the HPLC method, focusing on the analytical target product profile. Using the Design-Expert 13.0 software, we set up a CCD experimental design to explore the relationships between flow rate and buffer, looking at responses like retention time, peak area, and symmetry factor. This QbD-based HPLC method were examined and the peak purity of Rilpivirine with a UV detector, analysing all degradation products. The QbD method development proved to be more robust and efficient, delivering higher performance in a fraction of the time compared to traditional manual methods. Rilpivirine degrades under various conditions such as acidic, alkaline, oxidative, thermal, and photolytic and quantitatively analyzed these using HPLC method. The results showed the method was found to be stability indicating with great linearity, precision, accuracy, and limits of detection and quantification for Rilpivirine. This method stands out for its practicality and applicability. The QbD-developed method for Rilpivirine is not only efficient and cost-effective but also quick and stability-indicating, making it perfect for quality control labs.

FUNDING

No funds, grants, or other support was received.

COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies with human participants performed by any of the authors.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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TABLES

Table-1: Characteristics of the Solution Obtained from the Optimized Method

Flow Rate	Buffer%	Retention Time	Peak Area	Symmetry Factor
1 ml/min	40	3.3	5133891.79	1.6

Table-2: Design Matrix As per CCD

Run	Factor -1 A:Flow rate	Factor- 2 B:Buffer %	Response-1 Retention time	Response-2 Peak area	Response-3 Symmetry factor
1	0.8	40	4.183	5927276.5	1.48
2	1	40	3.342	5133891.79	1.45
3	1	40	3.342	5296253	1.44
4	1.2	40	2.767	4434524.5	1.40
5	1.2	40	2.775	4427263.75	1.38
6	0.8	50	5.225	6559323.26	1.36
7	1	50	4.192	5274582	1.32
8	1.2	50	3.533	4330585.2	1.35
9	1.2	50	3.525	4340942.25	1.36
10	0.8	60	9.142	6597382.25	1.38
11	1	60	7.208	5195133	1.36
12	1.2	60	6.025	440618.5	1.43
13	1.2	60	6.000	4381215.5	1.42

Table-3: Optimized Method Parameters

Flow rate	1 ml/min
Column	C18 column (5 µm particle size, L × I.D. 15 cm × 4.6 mm)
detector	UV detector
Detector wavelength	298 nm
Injection volume	10.0 µL
Run time	10 min

Table-4: Obtained Accuracy Results

Concentration levels	%Mean recovery
80%	98.88%
100%	108.15%
120%	100.75%

Table-5: Intraday precision at 298 nm

Concentration(ppm)	Peak Area	%RSD
30	3549141.93	0.029
	3697965.0	
	3756101.50	
40	5343608.65	0.880
	5195770.96	
	5407708.51	
50	5532575.06	0.562
	5598814.0	
	5502344.59	

Table-6: Inter day Precision at 298nm

Concentration	Peak Area	% RSD
30	3514068.1	1.33
	3601591.32	
	3517823.26	
40	5087575.87	1.07
	5087590.89	
	4975843.97	
50	5521788.78	1.003
	5467819.88	
	5578627.11	

Table-7: Summary Chart of Forced Degradation Studies

Type of forced degradation	Retention time of Actual peak (min)	Retention time of degraded molecule(min)	%degradation
Acid Degradation	3.01	5.32	13.04
Alkali Degradation	3.01	5.35	19.87
Oxidative Degradation	3.00	5.35	18.2
Thermal Degradation	3.06	5.34	19.7
Photolytic Degradation	3.06	5.34	15.2

FIGURES

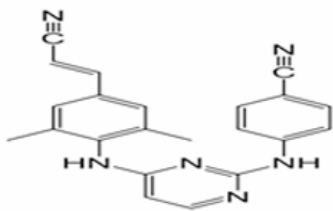


Fig. 1. Chemical Structure Of Rilpivirine

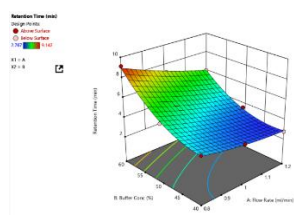


Fig. 2. 3D Plot For Retention Time

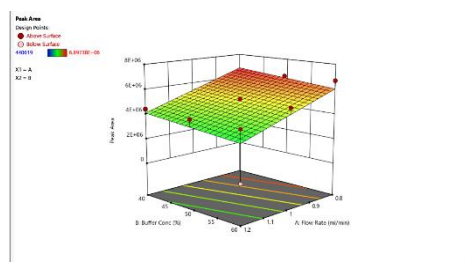


Fig. 3. 3D Surface plot For Peak Area

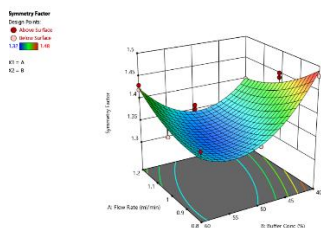


Fig. 4. 3D Surface Plot For Symmetry Factor

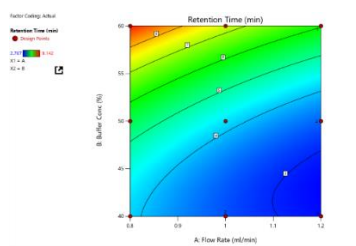


Fig. 5. 2D Contour plot of Retention time

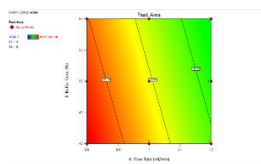


Fig. 6. 2D Contour Plot Of Peak area

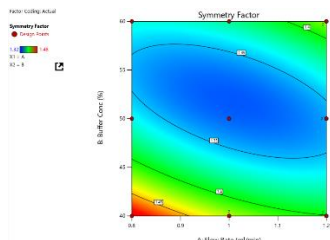


Fig. 7. 2D Contour Plot Of Symmetry factor

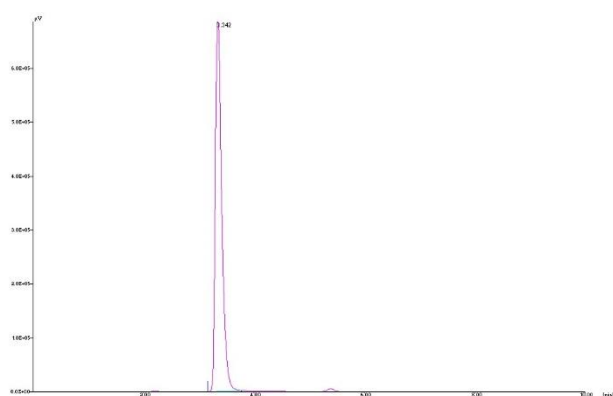


Fig. 8. Optimized Chromatogram Of Rilpivirine

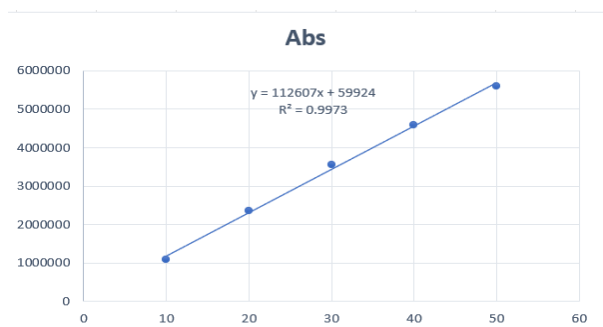


Fig. 9. Calibration curve of Linearity

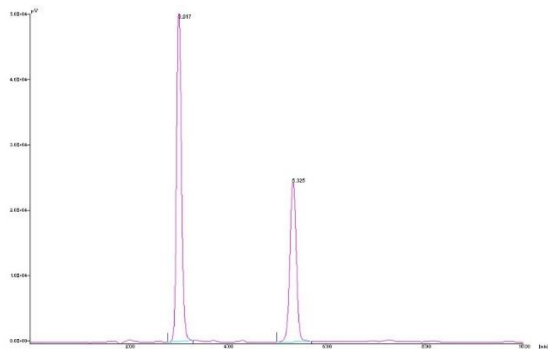


Fig. 10. Chromatogram Of Acid Degradation

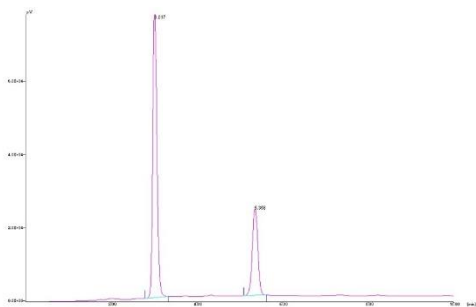


Fig. 11. Chromatogram Of Alkali Degradation

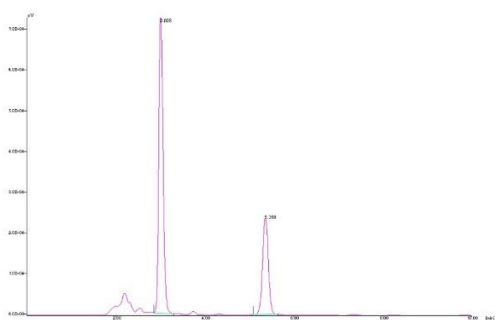


Fig. 12. Chromatogram Of Oxidative Degradation

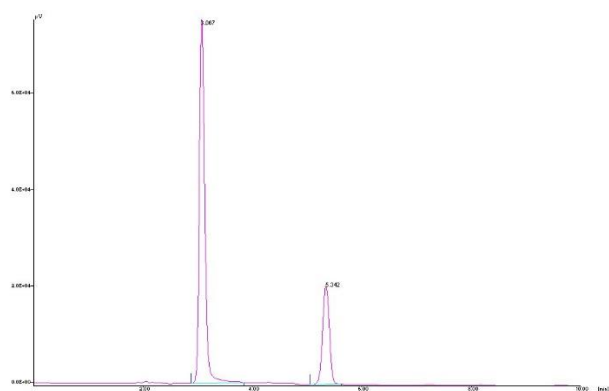


Fig. 13. Chromatogram Of Thermal Degradation

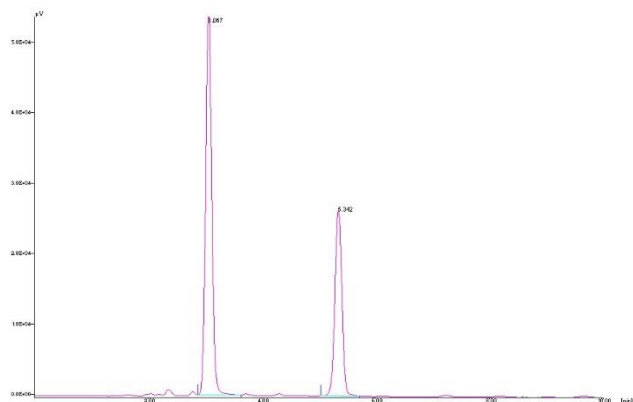


Fig. 14. Chromatogram Of Photolytic Degradation

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