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Research Paper

Systematic QBD Approach for Analytical Method Development and Validation of Rivaroxaban Using RP-HPLC Method

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ABSTRACT

This study presents a systematic Analytical Quality by Design (AQbD) approach for the development and validation of a robust Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) method for the quantitative estimation of Rivaroxaban in tablet dosage forms. Utilizing a Central Composite Design (CCD), the study systematically investigated the influence of critical method parameters—specifically mobile phase composition and flow rate—on chromatographic responses, including retention time, peak area, and theoretical plates. The experimental design and optimization were undertaken using Design-Expert® software, with statistical significance evaluated via Analysis of Variance (ANOVA). A mobile phase of Methanol:0.1% Acetic Acid (83.4:16.6, v/v) at a flow rate of 0.93 mL/min and detection at 248 nm made up the ideal chromatographic conditions. Excellent specificity, linearity (10–50 µg/mL; $R^2 > 0.999$), accuracy, precision, and robustness were demonstrated by the method, which was verified in accordance with International Council for Harmonization (ICH) Q2(R2) requirements. The assay of marketed tablet formulations was successfully conducted using the validated method, producing results that were in line with label claims. This study confirms that the AQbD-based RP-HPLC method is a simple, reliable, and reproducible tool suitable for routine pharmaceutical quality control, offering enhanced regulatory compliance and process understanding compared to traditional empirical methods.

INTRODUCTION

Rivaroxaban (RBN) is a novel, direct acting, target-specific, potent oral anticoagulant drug. By potentially reducing both free and clot-bound coagulation factor Xa (Vitamin K dependent plasma protein) and prothrombinase activity, it operates at a critical point in the blood-clotting process; hence, efficient inhibition of thrombin production results in an extension of the clotting

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time [1]. It is used to treat and prevent stroke in adult patients with atrial fibrillation, suppress cardiovascular events linked to acute coronary syndrome, and avoid venous thromboembolism in patients undergoing selected hip or knee replacement surgery [2]. RBN is a great substitute for low molecular weight heparins in the management and avoidance of pulmonary embolism and deep vein thrombosis linked to cancer [3].

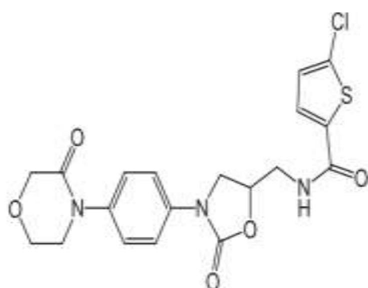

Figure 1. Chemical structure of rivaroxaban[23]

Selecting appropriate experimental circumstances to precisely quantify a medicine in the presence of excipients, contaminants, and degradation products is the main goal of analytical technique development. Robustness, reproducibility, accuracy, precision, and specificity are all desirable characteristics of an ideal analytical method [4]. The most used method for rivaroxaban analysis is reverse-phase high-performance liquid chromatography (RP-HPLC) because of its precision, accuracy, and repeatability [5]. For moderately polar medicinal compounds, RP-HPLC provides good separation, peak symmetry, and reproducibility [6].

Pharmaceutical QbD is a systematic approach to development that starts with predetermined goals and focuses on understanding and controlling products and processes using strong science and quality risk management [7].

The QbD approach must accurately map product attributes to process parameters by ensuring the product’s area of design ’, a space with several dimensions consisting of various qualities, is identified and explained.[8] Quality by design (QbD) in the production process can be linked to many concepts connected to similar notions in developing analytical methodologies [9]. The analytical target profile (ATP), which describes the goal of the measurement, is the first step in analytical QbD (AQbD). The crucial method parameters (CMPs), which are based on comprehensive analysis and evaluation of risk approaches, are then thoroughly examined to emphasize the significance of completely understanding the analytical system. The design space (DS) is the multidimensional area of the CMPs' successful operating ranges that generates the desired values for the critical method attributes (CMAs) [10].

2. MATERIALS AND METHODS

2.1 Materials

Rivaroxaban working standard was obtained as a gift sample from a prominent pharmaceutical producer and used without additional purification. Methanol (HPLC grade), acetic acid (analytical grade), and distilled water were employed throughout the investigation. For assay and validation investigations, commercially available rivaroxaban tablets with 20 mg of the medication were purchased from the neighborhood market.

2.2 Instrumentation

Chromatographic analysis was performed using an Agilent 1100 Series HPLC system equipped with a Diode Array Detector (DAD) and ChemStation software. Separation was achieved using an Agilent Eclipse XDB C18 column (250 mm × 4.6 mm, 5 µm particle size). A Shimadzu analytical balance was used for weighing, while sonication was carried out using an ultrasonic bath. All solutions were filtered through a 0.45 µm membrane filter prior to analysis [11].

Table 1. Instrumentation Used

Instrument	Specification
HPLC System	Agilent 1100 Series
Detector	DAD
Software	ChemStation
Column	Agilent Eclipse XDB C18
Column Dimension	250 × 4.6 mm
Particle Size	5 µm
Analytical Balance	Shimadzu
Sonicator	Ultrasonic Bath
Filter	0.45 µm Membrane Filter

2.3 Chromatographic Conditions

Using a mobile phase made up of methanol and 0.1% acetic acid, chromatographic separation was carried out using an Agilent Eclipse XDB C18 column. The composition of the optimized mobile phase was kept at 83.4:16.6 (v/v). Using a DAD detector, the flow rate was adjusted to 0.93 mL/min and detection was done at 248 nm. Chromatographic analysis was carried out under isocratic elution at room temperature using a 20 µL injection volume. RP-HPLC techniques have been shown to effectively separate and estimate rivaroxaban under similar chromatographic circumstances [12].

Table 2. Optimized Chromatographic Conditions

Parameter	Condition
Column	Agilent Eclipse XDB C18 (250 × 4.6 mm, 5 µm)
Mobile Phase	Methanol : 0.1% Acetic Acid (83.4 : 16.6 v/v)
Flow Rate	0.93 mL/min
Detection Wavelength	248 nm
Injection Volume	20 µL
Detector	DAD
Temperature	Ambient
Elution Mode	Isocratic

2.4 Preparation of Standard Solution

A 10 mg working standard was precisely weighed and then transferred into a 10 mL volumetric flask to create a stock solution containing 1000 µg/mL of rivaroxaban. The medication was dissolved in methanol and the volume was adjusted to the mark with the same solvent. For linearity and optimization experiments, working standard solutions of 10, 20, 30, 40, and 50 µg/mL were created by appropriately diluting the stock solution with the mobile phase [13,14].

2.5 Preparation of Sample Solution

Twenty rivaroxaban tablets were precisely weighed and ground into a fine powder. To acquire a concentration of 1000 µg/mL, a quantity of powder equal to 10 mg of rivaroxaban was put into a 10 mL volumetric flask, dissolved in the mobile phase, sonicated for full extraction, and diluted to volume using the same solvent. Prior to analysis, the solution was passed through a 0.45 µm membrane filter. An aliquot of the filtered stock solution was further diluted with mobile phase to

achieve a final concentration of 40 µg/mL for assay measurement [15,16].

3. SYSTEMATIC QBD APPROACH FOR METHOD DEVELOPMENT

3.1 Analytical Target Profile (ATP)

The Analytical Target Profile (ATP) was established to create an RP-HPLC approach for quantitatively estimating rivaroxaban in tablet dose forms that is straightforward, accurate, precise, robust, and dependable. Acceptable retention time, peak symmetry, column efficiency, and adherence to ICH validation standards were among the intended analytical outcomes. The AQbD architecture is based on ATP, which specifies the analytical method's performance standards and intended use [17].

Table 3. Analytical Target Profile

Parameter	Target Criteria
Retention Time	< 10 min
Tailing Factor	≤ 2.0
Theoretical Plates	> 2000
Precision (%RSD)	< 2.0
Accuracy (% Recovery)	98–102%

3.2 Risk Assessment

Analytical variables that could have a substantial impact on chromatographic performance were identified through risk assessment. Mobile phase composition and flow rate were found to be important factors influencing retention time, peak area, and theoretical plates based on findings from the literature and first experimental investigations. Risk assessment makes it easier to identify and control variables in a methodical way when developing and optimizing analytical methods [18].

3.3 Selection of Critical Method Attributes (CMAs) and Critical Method Parameters (CMPs)

The critical method attributes (CMAs) selected for examination were retention time, peak area, and theoretical plates. Due to their substantial impact on chromatographic responses, mobile phase composition and flow rate were chosen as crucial method parameters (CMPs) [19].

Table 4. Selected CMAs and CMPs

CMAs	CMPs
Retention Time	Mobile Phase Composition
Peak Area	Flow Rate
Theoretical Plates	Detection Wavelength

3.4 Central Composite Design (CCD)

Chromatographic factors were systematically optimized using a Central Composite Design (CCD). One of the most popular response surface methodology designs is CCD, which minimizes the number of experimental trials needed while assessing the impact of independent factors and their interactions on analytical answers [20]. The experimental design was created and examined using Design-Expert® software.

Table 5. Experimental Factors and Levels

Factor	Low (-1)	Center (0)	High (+1)
Methanol (%)	80.0	83.4	86.0
Flow Rate (mL/min)	0.80	0.93	1.10

3.5 Statistical Analysis and Optimization

Design-Expert® software was used to statistically assess the experimental results from CCD runs. Model significance and factor effects were assessed using analysis of variance (ANOVA). Relationships between chromatographic factors and analytical results were established using response surface methodology (RSM). Desirability function analysis was used in numerical optimization to find chromatographic conditions that would yield the best analytical performance [21].

4. METHOD VALIDATION

In terms of system appropriateness, specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, robustness, ruggedness, and assay, the optimized RP-HPLC technique was validated in accordance with the International Council for Harmonisation (ICH) Q2(R2) guideline [22].

4.1 System Suitability

A standard rivaroxaban solution was repeatedly injected to assess the appropriateness of the system before analysis. Retention duration, peak area, theoretical plates, and tailing factor were among the parameters assessed to confirm the chromatographic system's functionality. [22]

4.2 Specificity

By comparing the chromatograms of blank, standard, and sample solutions, specificity was assessed in order to identify any possible interference during the rivaroxaban retention period.(22)

4.3 Linearity

Plotting peak area against concentration and computing the regression equation and correlation coefficient allowed for the assessment of linearity over the concentration range of 10–50 µg/mL.[24]

4.4 Limit of Detection (LOD) and Limit of Quantification (LOQ)

In accordance with ICH guidelines, LOD and LOQ were calculated using the response's standard deviation and the calibration curve's slope.

4.5 Accuracy

Recovery trials at 80%, 100%, and 120% concentration levels using the usual addition method were used to assess accuracy.

4.6 Precision

Precision was tested in terms of repeatability (intraday precision) and intermediate precision (interday precision) and reported as percentage relative standard deviation (%RSD).[25]

4.7 Robustness

In order to assess robustness, tiny intentional changes were made to chromatographic settings, such as the composition of the mobile phase and the detection wavelength, and the impact on analytical performance was monitored.

4.8 Ruggedness

Ruggedness was evaluated by doing analysis on many days and under various operational conditions to ascertain technique repeatability.

4.9 Assay

The validated RP-HPLC method was utilized for quantitative measurement of rivaroxaban in marketed tablet formulations and the percentage assay was calculated.

5. RESULTS AND DISCUSSION

5.1 Optimization of Chromatographic Conditions

Preliminary chromatographic trials were performed using different mobile phase compositions and flow rates to obtain satisfactory separation of rivaroxaban. Different methanol and aqueous phase ratios were examined. Methanol:0.1% Acetic Acid (83.4:16.6, v/v) at a flow rate of 0.93 mL/min and a detection wavelength of 248 nm made up the ideal chromatographic conditions. Rivaroxaban showed a strong, symmetrical peak with acceptable retention time and column efficiency in these circumstances.

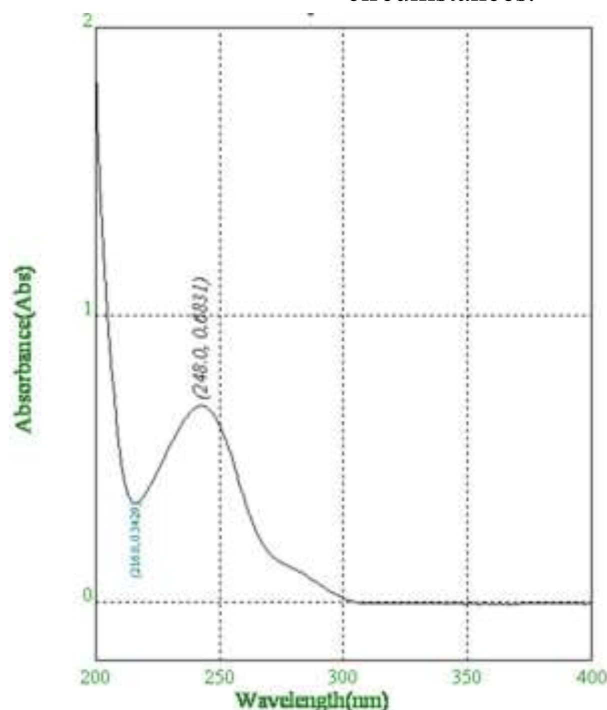

Figure 1. Optimized Standard Chromatogram

5.2 Central Composite Design (CCD) Studies

Central Composite Design (CCD) was used to examine how mobile phase composition (A) and flow rate (B) affected chromatographic results. Design-Expert® software produced 13 experimental runs in all, and the results were documented in terms of theoretical plates, peak area, and retention time.

Table 6. CCD Experimental Design Matrix

Std Run	Factor A: Mobile Phase (%)	Factor B: Flow Rate (mL/min)	Response 1(R1) : RT (min)	Response 2(R2): Area (AUC)	Response 3(R3) : TP (Theoretical Plates)
1	80.00	1.14	3.486	1066.66	12941
2	85.00	0.90	4.415	1397.03	13978
3	80.00	1.00	4.001	1218.61	13736
4	85.00	1.10	3.617	1116.75	13475
5	80.00	1.00	4.002	1226.81	13654
6	80.00	0.86	4.778	1464.91	15228
7	80.00	1.00	4.009	1237.38	13725

Std Run	Factor A: Mobile Phase (%)	Factor B: Flow Rate (mL/min)	Response 1(R1) : RT (min)	Response 2(R2): Area (AUC)	Response 3(R3) : TP (Theoretical Plates)
8	75.00	0.90	4.678	1367.70	14150
9	87.07	1.00	3.969	1244.61	12662
10	80.00	1.00	4.007	1229.46	13722
11	80.00	1.00	4.008	1238.69	13726
12	75.00	1.10	3.784	1106.35	12448
13	72.93	1.00	4.272	1231.88	12827

5.3 ANOVA Analysis for Retention Time

With a p-value of less than 0.05, the quadratic model produced for retention time was determined to be significant, suggesting that the chosen model sufficiently explained the connection between independent variables and retention time. The anticipated and observed responses showed good agreement, as indicated by the coefficient of determination (R^2).

Table 7. ANOVA for Retention Time (R1)

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	1.69	5	0.3373	1003.79	<0.0001
A – Mobile Phase	0.0921	1	0.0921	274.16	<0.0001
B – Flow Rate	1.55	1	1.55	4606.82	<0.0001
AB	0.0023	1	0.0023	6.86	0.0345
A^2	0.0225	1	0.0225	66.94	<0.0001
B^2	0.0273	1	0.0273	81.16	<0.0001
Residual	0.0024	7	0.0003	–	–
Lack of Fit	0.0023	3	0.0008	57.62	0.0010
Pure Error	0.0001	4	0.0000	–	–
Cor Total	1.69	12	–	–	–

Model Statistics

Parameter	Value
R^2	0.9986
Adjusted R^2	0.9976
Predicted R^2	0.9903
Adeq Precision	99.9071
C.V. (%)	0.4494

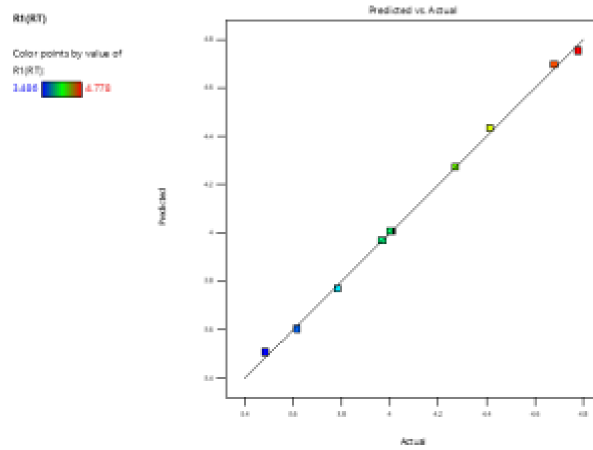

Figure 2. Predicted versus Actual plot for Retention Time (R1) showing excellent agreement between experimental and predicted values.

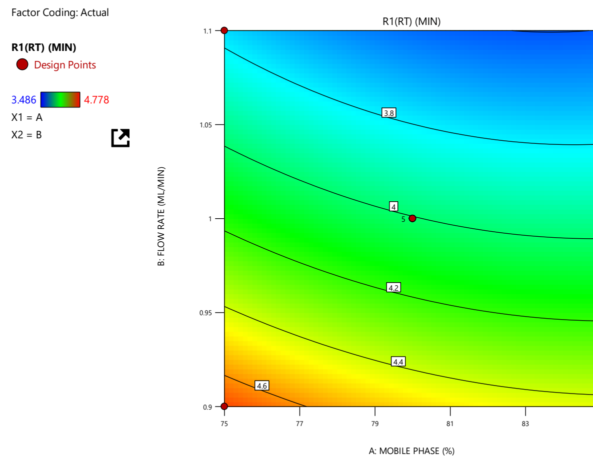

Figure 3. Contour plot illustrating the combined effect of mobile phase composition and flow rate on retention time.

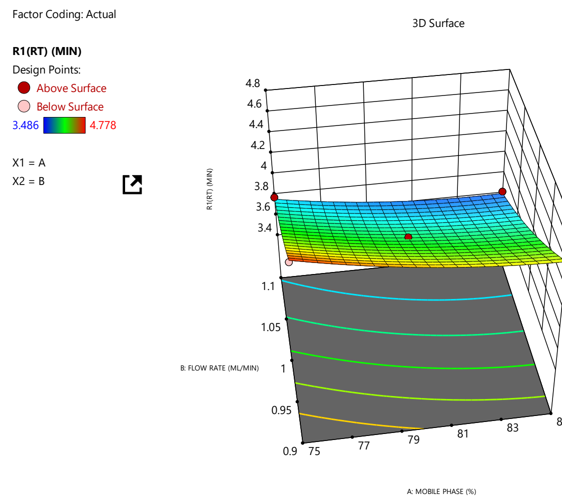

Figure 4. Three-dimensional response surface plot showing the influence of mobile phase composition and flow rate on retention time.

5.4 ANOVA Analysis for Peak Area

Response surface methodology was used to assess how mobile phase composition and flow rate affected peak area. The developed model was found to be significant and to provide a sufficient explanation for the observed variability in peak area based on statistical analysis.

Table 8. ANOVA for Peak Area (R2)

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	1.550E+05	5	30999.07	495.11	<0.0001
A – Mobile Phase	416.52	1	416.52	6.65	0.0365
B – Flow Rate	1.526E+05	1	1.526E+05	2437.03	<0.0001
AB	89.65	1	89.65	1.43	0.2704
A^2	53.07	1	53.07	0.8477	0.3878
B^2	1901.99	1	1901.99	30.38	0.0009
Residual	438.28	7	62.61	–	–
Lack of Fit	168.31	3	56.10	0.8313	0.5421
Pure Error	269.96	4	67.49	–	–
Cor Total	1.554E+05	12	–	–	–

Model Statistics

Parameter	Value
R^2	0.9972
Adjusted R^2	0.9952
Predicted R^2	0.9896
Adeq Precision	72.6652
C.V. (%)	0.6371

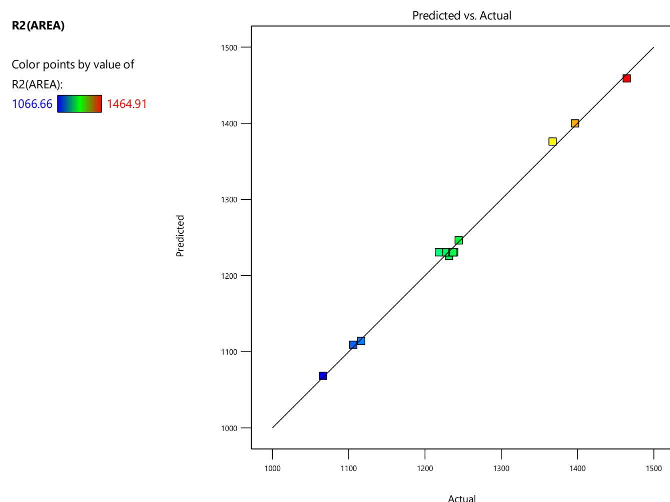

Figure 5. Predicted versus Actual plot for Peak Area showing good agreement between experimental and predicted responses.

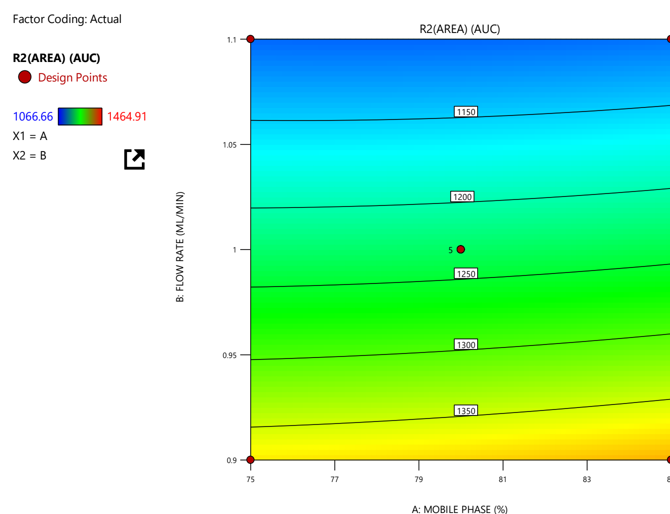

Figure 6. Contour plot illustrating the combined effect of mobile phase composition and flow rate on peak area.

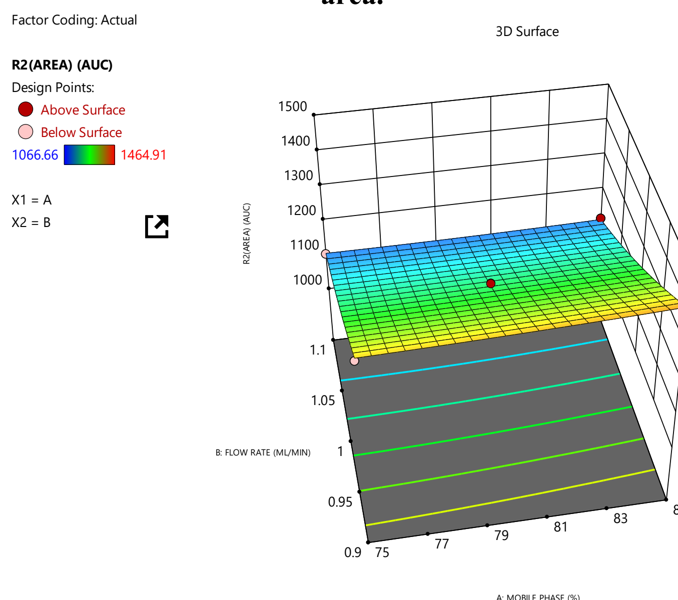

Figure 7. Three-Dimensional Surface Plot for Peak Area

5.5 ANOVA Analysis for Theoretical Plates

As a measure of chromatographic efficiency, theoretical plates were chosen. The constructed quadratic model showed a substantial impact of certain chromatographic variables on column efficiency and a reasonable prediction ability.

Table 9. ANOVA for Theoretical Plates

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	5.12E+07	5	1.02E+07	76.52	<0.0001
A – Mobile Phase	1.53E+06	1	1.53E+06	11.47	0.0118
B – Flow Rate	3.87E+07	1	3.87E+07	290.11	<0.0001
AB	1.82E+06	1	1.82E+06	13.64	0.0077
A^2	4.31E+06	1	4.31E+06	32.30	0.0008
B^2	3.12E+06	1	3.12E+06	23.40	0.0019

Source	Sum of Squares	df	Mean Square	F-value	p-value
Residual	9.34E+05	7	1.33E+05	–	–
Lack of Fit	6.87E+05	3	2.29E+05	3.71	0.118
Pure Error	2.47E+05	4	6.18E+04	–	–
Cor Total	5.21E+07	12	–	–	–

Model Statistics

Parameter	Value
R^2	0.9821
Adjusted R^2	0.9693
Predicted R^2	0.9425
Adeq Precision	28.41
C.V. (%)	1.87

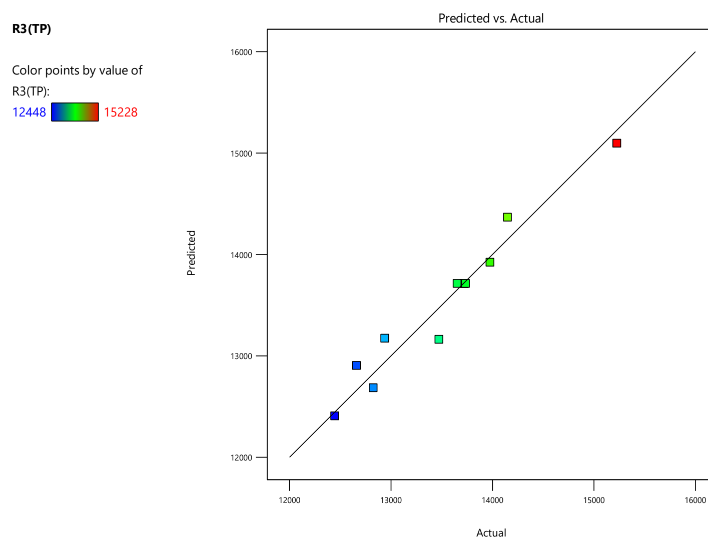

Figure 8. Predicted versus Actual plot for theoretical plates showing good correlation between experimental and predicted responses.

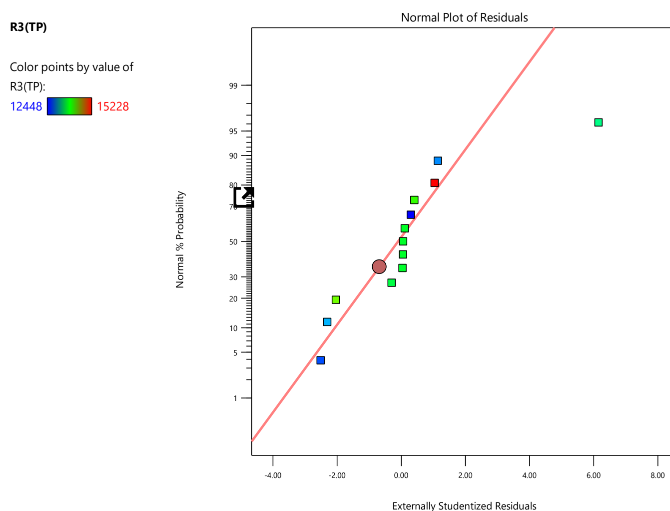

Figure 9. Normal Probability Plot of Residuals

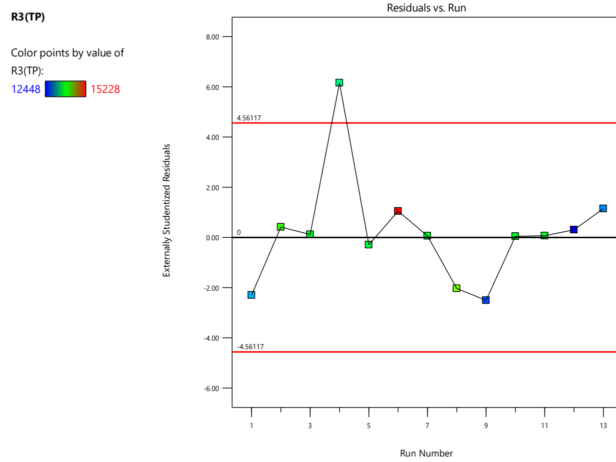

Figure 10. Residuals versus Run Plot

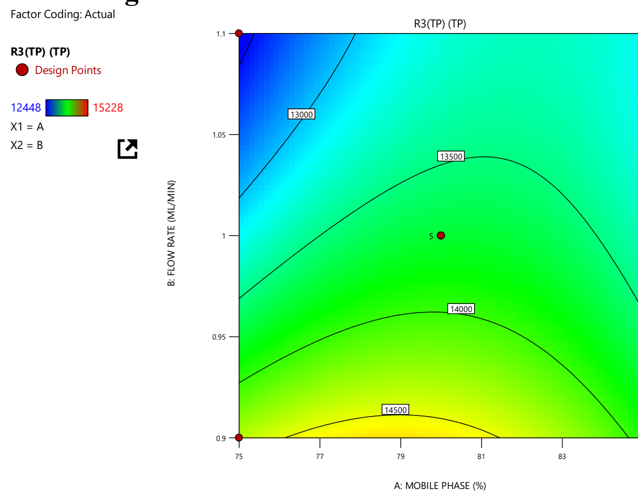

Figure 11. Contour Plot for Theoretical Plates

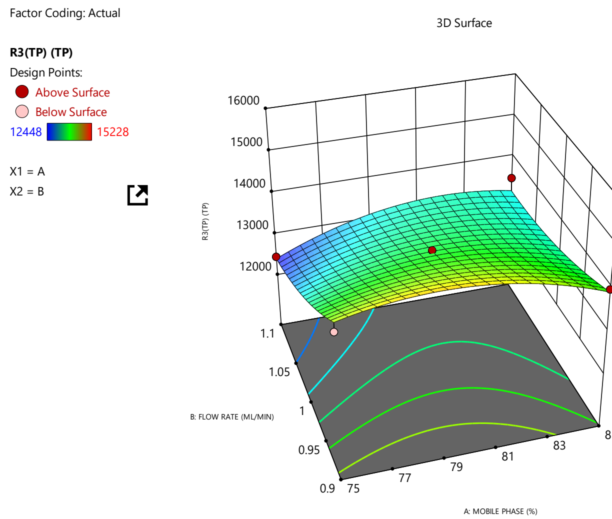

Figure 12. Three-Dimensional Surface Plot for Theoretical Plates

5.6 Polynomial Equations

The relationship between chromatographic variables and analytical responses was expressed

using second-order polynomial equations generated by Design-Expert® software.

Table 10. Polynomial Equations Generated by CCD

Response	***Polynomial Equation***
Retention Time (R1)	$RT = 4.24 - 0.11A - 0.44B + 0.024AB + 0.073A^2 + 0.080B^2$
Peak Area (R2)	$Peak\ Area = 1332.34 + 7.22A - 138.05B + 4.73AB + 3.62A^2 - 21.71B^2$
Theoretical Plates (R3)	$TP = 13929.40 + 438.25A - 2200.37B + 476.50AB - 1021.46A^2 - 869.38B^2$

Where:

- *A = Mobile Phase Composition (% Methanol)*
- *B = Flow Rate (mL/min)*

5.7 Numerical Optimization

Desirability function analysis was used in numerical optimization to find chromatographic conditions that could simultaneously meet all analytical goals. The optimal conditions suggested by the software comprised of a mobile phase composition of 83.4542% methanol and a flow rate of 0.936867 mL/min. Retention time of 4.25061 minutes, peak area of 1332.34, and theoretical plates of 13929.4 with an overall desirability value of 1.000 were the expected responses under these circumstances.

Table 11. Optimized Chromatographic Conditions and Predicted Responses

Factor/Response	Optimized Value
Mobile Phase (% Methanol)	83.4542
Flow Rate (mL/min)	0.936867
Retention Time (min)	4.25061
Peak Area	1332.34
Theoretical Plates	13929.4
Overall Desirability	1.000

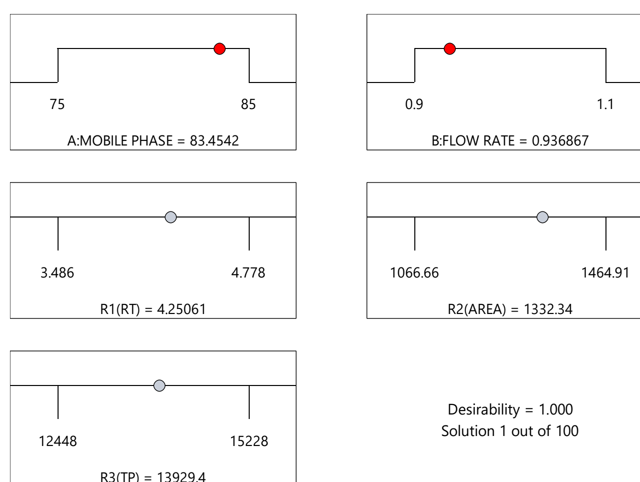

Figure 13. Desirability Ramp Plot generated by Design-Expert® software showing the optimized chromatographic conditions of methanol concentration (83.4542%) and flow rate (0.936867 mL/min) with predicted retention time (4.25061 min), peak area (1332.34), theoretical plates (13929.4), and overall desirability of 1.000.

5.8 Design Space and Overlay Plot

Desirability analysis and response surface methodology were used to create the design space. The area where all chromatographic results concurrently satisfied the predetermined acceptance criteria was shown by the overlay plot. The designed RP-HPLC method's robustness and dependability were validated by the acquired design space.

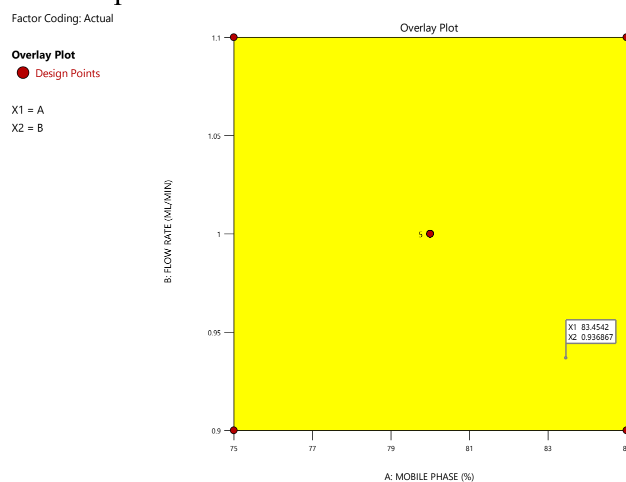

Figure 14. Overlay Plot

5.9 Method Validation Results

The optimized RP-HPLC method was validated according to ICH Q2(R2) guidelines.

5.9.1 System Suitability

System suitability parameters including retention time, peak area, theoretical plates, and tailing factor were found to be within acceptable limits, demonstrating satisfactory performance of the chromatographic system.

Table 12. System Suitability Results

Parameter	Injection 1	Injection 2	Mean Value	Acceptance Criteria
Retention Time (min)	4.229	4.230	4.230	< 10 min
Peak Area (mAU·s)	1678.77	1677.56	1678.17	%RSD ≤ 2.0
Theoretical Plates	13345	13347	13346	> 2000
Tailing Factor	0.93	0.93	0.93	≤ 2.0

System suitability parameters of the optimized RP-HPLC method for rivaroxaban. All evaluated parameters complied with the predefined acceptance criteria.

5.9.2 Specificity

No interfering peaks were observed at the retention time of rivaroxaban in blank chromatograms, confirming the specificity of the developed method.

Table 13. Specificity Results

Parameter	Chromatogram 1	Chromatogram 2
Retention Time (min)	4.252	4.255
Peak Area (mAU·s)	1676.49	1677.35
Symmetry Factor	0.96	0.96
Theoretical Plates	13249	13249
Interfering Peaks	Not Observed	Not Observed

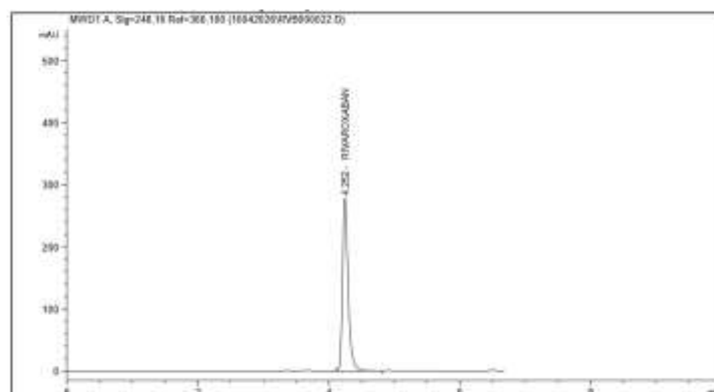

Figure 15. Representative specificity chromatogram of Rivaroxaban (40 µg/mL) showing a sharp and symmetrical peak at a retention time of 4.252 min with no interfering peaks, confirming the specificity of the developed RP-HPLC method.

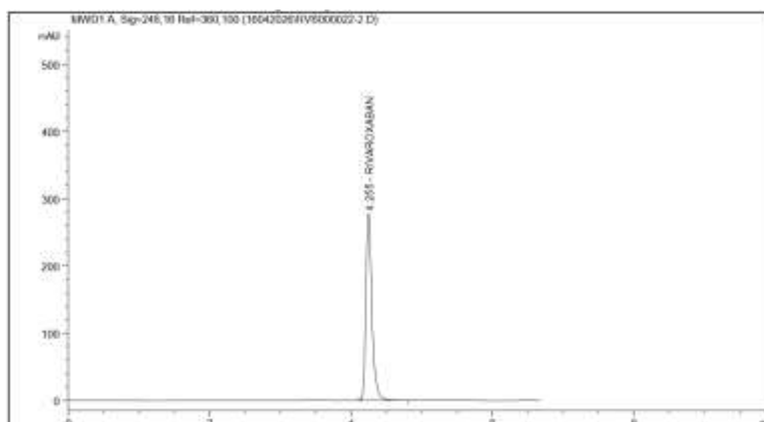

Figure 16. Replicate specificity chromatogram of Rivaroxaban (40 µg/mL) showing a sharp and symmetrical peak at a retention time of 4.255 min without any interfering peaks, confirming the selectivity and reproducibility of the developed RP-HPLC method.

5.9.3 Linearity

The method exhibited good linearity over the concentration range of 10–50 µg/mL with a correlation coefficient (R^2) greater than 0.999.

Table 14. Linearity Data

Concentration (µg/mL)	Mean Peak Area ± SD	%RSD
10	399.68 ± 1.52	0.38
20	858.06 ± 6.19	0.72
30	1252.96 ± 2.56	0.20
40	1674.42 ± 1.34	0.08
50	2132.99 ± 0.27	0.01

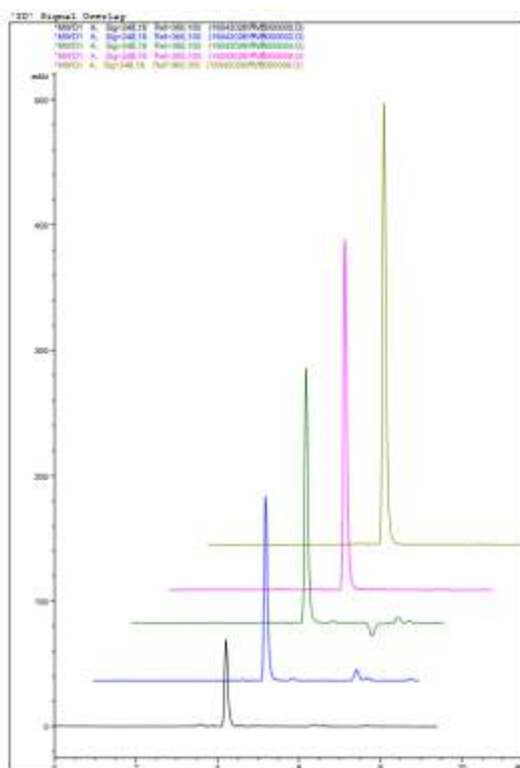

Figure 17. Overlay chromatograms of Rivaroxaban standard solutions showing chromatographic responses at different concentration levels. The overlaid chromatograms demonstrate consistent peak shape, retention behavior, and detector response across the studied concentration range.

5.9.4 LOD and LOQ

The calculated values of Limit of Detection (LOD) and Limit of Quantification (LOQ) demonstrated adequate sensitivity of the developed RP-HPLC method.

Table 15. LOD and LOQ Results

Parameter	Value (µg/mL)
Limit of Detection (LOD)	0.183
Limit of Quantification (LOQ)	0.554

5.9.5 Accuracy

Recovery studies performed at 80%, 100%, and 120% levels demonstrated satisfactory accuracy with percentage recovery within the acceptable range of 98–102%.

Table 16. Accuracy Results

Recovery Level (%)	Amount Added (µg/mL)	Amount Recovered (µg/mL)	% Recovery
80	8	7.95	99.38
100	10	9.98	99.80
120	12	12.10	100.83

Accuracy study results obtained using the standard addition method. Recovery values at all three concentration levels complied with ICH acceptance criteria (98–102%), confirming method accuracy.

5.9.6 Precision

The method showed acceptable repeatability and intermediate precision with %RSD values below 2.0%.

Table 17. Intraday Precision Results

Concentration (µg/mL)	Average Peak Area	%RSD
10	402.64	0.63
30	1255.65	0.13
50	2142.81	0.13

Table 18. Interday Precision Results

Concentration (µg/mL)	Average Peak Area	%RSD
10	402.30	0.39
30	1255.51	0.19
50	2149.06	0.01

5.9.7 Robustness

Minor deliberate changes in chromatographic conditions did not significantly affect analytical performance, indicating robustness of the method.

Table 19. Robustness Results

Parameter Varied	Condition	Retention Time (min)	Peak Area	Observation
Flow Rate	0.9 mL/min	4.52	1685.34	Acceptable
Flow Rate	1.0 mL/min (Optimized)	4.25	1678.17	Acceptable
Flow Rate	1.1 mL/min	3.98	1669.82	Acceptable
Mobile Phase (% Methanol)	82%	4.38	1682.45	Acceptable
Mobile Phase (% Methanol)	83% (Optimized)	4.25	1678.17	Acceptable
Mobile Phase (% Methanol)	84%	4.12	1671.93	Acceptable

5.9.8 Ruggedness

The developed method demonstrated reproducible results under different operating conditions and on different days.

Table 20. Ruggedness Results

Concentration (µg/mL)	Peak Area	Amount Found (µg/mL)	% Label Claim
40	1679.942	39.720	99.30
40	1678.690	39.691	99.23
Mean	1679.32	39.71	99.26
SD	0.885	0.021	0.052
%RSD	0.053	0.052	0.052

The developed RP-HPLC technique for rivaroxaban yielded robustness results under typical laboratory conditions. The low %RSD values (<2.0%) suggest great repeatability and robustness of the analytical procedure.

5.9.9 Assay

The assay of marketed rivaroxaban tablets was found to be within pharmacopeial limits, confirming suitability of the developed method for routine quality control analysis.

Table 21. Assay Results

Concentration (µg/mL)	Peak Area	Amount Found (µg/mL)	% Label Claim
40	1687.650	39.900	99.75
40	1682.150	39.772	99.43
Mean	**1684.90**	**39.84**	**99.59**
SD	**3.889**	**0.091**	**0.227**
%RSD	**0.231**	**0.228**	**0.228**

CONCLUSION

A systematic Analytical Quality by Design (AQbD)-based RP-HPLC method was successfully developed, optimized, and validated for the quantitative estimation of rivaroxaban in tablet dosage forms. The impact of crucial technique factors, such as flow rate and mobile phase composition, on chromatographic responses was successfully examined using Central Composite Design (CCD). ANOVA statistical examination verified the created models' validity and significance. Desirability function analysis and numerical optimization made it possible to create ideal chromatographic conditions that produced acceptable theoretical plates, peak area, and retention time.

The developed technique showed satisfactory specificity, linearity, accuracy, precision, robustness, sensitivity, and assay performance after being verified in accordance with ICH Q2(R2) requirements. The results confirmed that the proposed RP-HPLC method is simple, reliable, reproducible, and suitable for routine quality control analysis of rivaroxaban in pharmaceutical dosage forms. Additionally, the effective implementation of AQbD principles improved method comprehension, robustness, and regulatory compliance, making the developed analytical approach appropriate for industrial and pharmaceutical quality assurance applications.

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