



QbD Based RP-HPLC Method Development and Validation of Rivaroxaban in Bulk and Pharmaceutical Formulation

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Abstract: A simple, rapid, selective, and reproducible reversed-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the quantitative estimation of Rivaroxaban in tablet dosage forms. The chromatographic separation was performed using a Waters XBridge C18 column (250 mm × 4.6 mm, 5 µm particle size). A mixture of acetonitrile and water in a ratio of 60:40 (v/v) was used as the mobile phase at a flow rate of 1.1 mL/min. Detection was carried out at a wavelength of 250 nm, and the column temperature was maintained at ambient conditions. The retention time of Rivaroxaban was found to be 5.458 minutes. The method demonstrated excellent linearity over the concentration range of 2–12 µg/mL, with a correlation coefficient of 0.9999, indicating a strong linear relationship between concentration and chromatographic response. Method precision was evaluated, and the percentage relative standard deviation (%RSD) values were found to be less than 2% at all selected concentrations, confirming the reproducibility of the developed method. Accuracy was assessed through recovery studies, which yielded a mean percentage recovery of 99.50%, demonstrating satisfactory agreement between the measured and expected drug concentrations. The assay results for Rivaroxaban in the commercially available tablet formulation were within the acceptable range of 98–102%. The method was validated in accordance with the applicable International Council for Harmonization (ICH) guidelines to establish its suitability for pharmaceutical analysis. The results indicate that the developed RP-HPLC method offers satisfactory linearity, precision, accuracy, and reproducibility under the specified chromatographic conditions. Therefore, the proposed method can be successfully applied to the routine quantitative analysis and quality control of Rivaroxaban in bulk drug and tablet dosage forms, subject to appropriate validation of specificity and other relevant performance characteristics.

Keywords: Rivaroxaban, RP-HPLC, Method development, Method validation, Pharmaceutical analysis, Tablet dosage form.

1. Introduction

Rivaroxaban is an orally administered, direct-acting anticoagulant that selectively inhibits activated coagulation factor X (factor Xa). Factor Xa plays a crucial role in the coagulation cascade by catalyzing the conversion of prothrombin to thrombin, which subsequently promotes the formation of fibrin and the development of blood clots. By inhibiting factor Xa, Rivaroxaban reduces thrombin generation and prevents thrombus formation. It is used for the prevention and treatment of thromboembolic disorders, including venous thromboembolism, and for reducing the risk of stroke and systemic embolism in eligible patients with non-valvular atrial fibrillation. Its oral administration and predictable pharmacological profile have established Rivaroxaban as an important therapeutic option for anticoagulant treatment [1,2].



The increasing clinical use of Rivaroxaban necessitates reliable analytical methods for the identification, quantification, and quality control of the active pharmaceutical ingredient (API) and its finished pharmaceutical formulations. Accurate determination of drug content is essential to ensure dosage uniformity, product quality, batch-to-batch consistency, and compliance with established pharmaceutical standards. Analytical methods used for routine quality control should demonstrate appropriate selectivity, accuracy, precision, linearity, robustness, and sensitivity. These characteristics are particularly important for ensuring reliable measurements in bulk drug substances and complex pharmaceutical matrices [3].

High-performance liquid chromatography (HPLC), particularly reversed-phase HPLC (RP-HPLC), is widely employed in pharmaceutical analysis because of its versatility, reproducibility, separation efficiency, and compatibility with a broad range of pharmaceutical compounds. In RP-HPLC, separation is primarily governed by the differential interactions of analytes with a non-polar stationary phase and a relatively polar mobile phase. The technique permits controlled separation through the optimization of chromatographic parameters such as mobile-phase composition, pH, flow rate, detection wavelength, column chemistry, and column temperature. Consequently, RP-HPLC is suitable for the quantitative estimation of Rivaroxaban in bulk materials and finished dosage forms. However, conventional method development often relies on a trial-and-error approach, which may require considerable experimental effort and may not adequately establish the relationship between critical method parameters and analytical performance. Quality by Design (QbD) is a systematic, science-based approach that emphasizes understanding the relationship between process variables and product quality. In analytical chemistry, this concept is commonly referred to as Analytical Quality by Design (AQbD). The AQbD approach involves defining the analytical objective, identifying critical analytical attributes, assessing potential sources of variability, and optimizing the method through structured experimentation. The International Council for Harmonization (ICH) guideline Q14 provides a framework for science- and risk-based analytical procedure development, while ICH Q2(R2) describes the principles for demonstrating that an analytical procedure is fit for its intended purpose [3,4].

Unlike conventional trial-and-error development, AQbD encourages the systematic investigation of critical method parameters and their influence on chromatographic performance. For an RP-HPLC method, potentially critical method parameters include the organic solvent proportion, mobile-phase pH, flow rate, column temperature, and detection wavelength, depending on the chromatographic system. Critical analytical attributes may include retention time, peak area, peak symmetry, theoretical plate count, resolution, and percentage assay. Risk assessment tools, such as Ishikawa diagrams and failure mode and effects analysis (FMEA), can help identify parameters that require experimental investigation. Design of experiments (DoE), including factorial designs and response surface methodology, can subsequently be used to evaluate the individual and combined effects of selected parameters and identify suitable operating conditions [3,5].

A systematic AQbD strategy can establish a scientifically justified method-operable region in which acceptable analytical performance is maintained despite reasonable variations in method parameters. This understanding can improve method robustness, reduce unnecessary experimental trials, and support consistent performance during routine quality control. The approach also provides a structured basis for analytical procedure monitoring and lifecycle management [3,5].

Although conventional RP-HPLC methods have been reported for the estimation of rivaroxaban, the application of a QbD-based strategy provides an opportunity to systematically evaluate chromatographic variables, understand their influence on analytical performance, and establish a robust method for routine pharmaceutical analysis. A recent publication has also reported an Analytical Quality by Design-based green RP-HPLC method for rivaroxaban, highlighting the importance of assessing the existing literature carefully when establishing the novelty of a new study [6].



Therefore, the present study aims to develop and validate a QbD-based RP-HPLC method for the quantitative estimation of rivaroxaban in bulk drug and pharmaceutical tablet formulations. The proposed approach involves defining the analytical target profile, identifying critical method parameters and analytical attributes, performing risk assessment, and optimizing the chromatographic conditions using an appropriate experimental design. The optimized method is intended to be evaluated for relevant performance characteristics, including specificity, linearity, accuracy, precision, robustness, and system suitability, in accordance with ICH Q2(R2) and the development principles described in ICH Q14 [3,4]. The overall objective is to establish a reliable, reproducible, and scientifically justified analytical procedure suitable for routine quality-control applications.

2. Materials and Methods

Rivaroxaban active pharmaceutical ingredient (API) and commercially available Xarelto® 15 mg tablets were used as the drug samples. HPLC-grade acetonitrile and methanol were used as organic solvents. Potassium dihydrogen phosphate, sodium hydroxide, and distilled water were used for the preparation of the mobile phases and pH adjustment, wherever required.

Chromatographic analysis was performed using a Shimadzu LC-2030 high-performance liquid chromatography (HPLC) system equipped with a Waters XBridge C18 analytical column (250 × 4.6 mm internal diameter, 5 µm particle size). Chromatographic data acquisition and processing were performed using LabSolutions software. A calibrated pH meter (Lab India) was used for pH measurement, and a precision analytical balance (Mettler Toledo) was used for weighing the chemicals and drug samples. A sonicator (Roxol) was used for degassing solutions and facilitating sample preparation, as required[7].

3. Preliminary Analysis of Rivaroxaban

Organoleptic Characteristics

The physical appearance of rivaroxaban API, including its colour and texture, was examined visually and compared with the reported characteristics available in established drug information resources, including DrugBank. This preliminary examination was performed to assess the general appearance of the drug sample before further analysis.

Solubility Studies

The solubility of rivaroxaban was qualitatively evaluated in selected solvents, including dimethyl sulfoxide (DMSO), methanol, ethanol, and acetonitrile. A small quantity of the drug was added separately to each solvent, and the mixtures were examined for dissolution under comparable experimental conditions. The observations were recorded to identify suitable solvents for preparing the drug stock solution and subsequent chromatographic analysis.

UV Spectrophotometric Analysis

The UV absorption characteristics of rivaroxaban were investigated by scanning an appropriately prepared drug solution over the wavelength range of 200–400 nm using a UV-visible spectrophotometer. The absorption spectrum was recorded to identify the wavelength of maximum absorption (λ_{max}), which could be considered for subsequent analytical investigations. The selected analytical wavelength should be confirmed experimentally before its use in HPLC method development[8].

1. Experimental Design and Optimization

Design of Experiments

A design of experiments (DoE) approach was employed to systematically investigate the influence of chromatographic variables on the performance of the proposed RP-HPLC method. The experimental design was



generated using Design-Expert® software, version 8. The optimal design approach was selected to evaluate the effects of selected experimental factors while minimizing the number of experimental runs required to obtain meaningful information about the chromatographic responses. The design was intended to facilitate the systematic evaluation of mobile-phase composition and pH and to identify chromatographic conditions that provide satisfactory separation and peak characteristics for rivaroxaban.

Selection of Chromatographic Factors and Responses

A Waters XBridge C18 column (250 × 4.6 mm, 5 µm) was selected as the stationary phase for method development. The following experimental factors and chromatographic responses were considered.

Independent variables (formulation of experimental conditions):

1. Organic-phase proportion in the mobile phase.
2. pH of the mobile phase.

Dependent variables (chromatographic responses):

1. Retention time.
2. Peak area.
3. Theoretical plate number.
4. Peak asymmetry.

The independent variables were varied according to the experimental design, while the dependent variables were measured for each experimental run. Retention time was assessed to determine the elution behaviour of rivaroxaban, peak area was used to evaluate the chromatographic response, theoretical plate number was considered an indicator of column efficiency, and peak asymmetry was used to assess peak shape.

Selection of Mobile Phases

Two mobile-phase systems were investigated during method development:

- Acetonitrile–water.
- Methanol–water.

For each mobile-phase system, the organic-phase proportion was investigated over the range of 40–60% (v/v), and the mobile-phase pH was varied between 6.5 and 7.5. The aqueous phase and pH-adjustment procedure were maintained consistently within each experimental series. Where required, potassium dihydrogen phosphate buffer and sodium hydroxide were used for pH adjustment, according to the experimental protocol.

The selected ranges were entered into Design-Expert® software to generate an optimal experimental design for each mobile-phase system[8].

Execution of the Experimental Design

For the acetonitrile–water mobile-phase system, the software generated nine experimental runs involving different combinations of organic-phase proportion and mobile-phase pH within the predefined ranges. Each run was performed using the selected C18 column, and the resulting chromatograms were evaluated for retention time, peak area, theoretical plate number, and peak asymmetry.

The same experimental design procedure was subsequently applied to the methanol–water mobile-phase system. Nine additional runs were performed for this system. Thus, a total of 18 experimental runs were planned across the two mobile-phase systems, with nine runs allocated to each system.

The chromatographic responses obtained from each run were entered into the software for statistical evaluation and optimization. The influence of the selected factors on the responses was assessed, and the predicted optimum chromatographic conditions were identified based on the optimization criteria.

Optimization of Chromatographic Conditions

The optimization procedure was performed to identify a combination of mobile-phase composition and pH that provided satisfactory chromatographic performance. The optimization criteria were established by considering the desired retention time, adequate peak area, higher theoretical plate number, and acceptable peak symmetry.

The optimum conditions predicted by the software were to be verified experimentally by performing confirmatory chromatographic runs. The experimental responses obtained under the optimized conditions should be compared with the predicted values to assess the suitability of the developed method.

The optimized chromatographic conditions were subsequently intended for further evaluation of the analytical performance of the proposed RP-HPLC method for Rivaroxaban in bulk drug and tablet dosage forms [9].

Table1: Runs were suggested by the software

Sr. No	Mobile Phase Composition (Organic Phase)	pH of Buffer
1	40	6.5
2	60	6.5
3	60	7.5
4	40	7.5
5	60	6.5
6	40	7.5
7	40	6.5
8	60	7.5
9	40	7.5

Preparation of the Mobile Phase

A total of 1000 mL of water was adjusted to pH 7.5 using dilute triethylamine. The mobile phase was prepared by mixing the pH-adjusted water with acetonitrile in a ratio of 40:60 (v/v). The resulting mobile phase was filtered and degassed before use in the HPLC system [10].

Preparation of the Rivaroxaban Stock Solution

A stock solution of rivaroxaban was prepared by accurately weighing 10 mg of rivaroxaban API and dissolving it in acetone. The solution was then diluted to a final volume of 10 mL with methanol in a volumetric flask to obtain a concentration of 1000 µg/mL. Subsequently, 0.1 mL of this stock solution was transferred into a 10 mL volumetric flask and diluted to the mark with methanol to obtain a final concentration of 10 µg/mL. This solution was labelled as the standard rivaroxaban solution.

Selection of the Detection Wavelength

An appropriate dilution of the standard rivaroxaban solution was prepared using water and scanned over the wavelength range of 200–400 nm using a UV-visible spectrophotometer. The resulting absorption spectra were recorded and overlaid to identify a suitable detection wavelength. Rivaroxaban exhibited considerable absorbance at 250 nm; therefore, this wavelength was selected for subsequent HPLC analysis [11].

Optimization- Screening design for suitable chromatographic condition:

Determination of suitable chromatographic conditions is based on peak parameters. Methanol: Water, ACN: Water, these two mobile phases were selected for screening study on C18 columns at pH 6.5 and 7.5. These mobile phases were screened by varying the organic phase composition from 40 to 60 % v/v and flow rate 1.10 mL/ min

Table 2 Optimized trials suggested by software based on desirability value

Sr. no.	Amount of Methanol	pH of AQ	Retention time	Tailing factor	Theoretical plates	Desirability
1	60	7.5	5.587	0.957	27747	0936

Optimized chromatographic conditions

The optimized chromatographic conditions for the analysis of Rivaroxaban were established using a reversed-phase high-performance liquid chromatography (RP-HPLC) system. The mobile phase consisted of acetonitrile and water in a ratio of 60:40 (v/v), with the aqueous phase adjusted to pH 7.5. Chromatographic separation was performed using a Waters XBridge C18 analytical column (250 × 4.6 mm, 5 µm particle size). UV detection was carried out at 250 nm. The injection volume was 10 µL, the flow rate was maintained at 1.10 mL/min, and the column temperature was maintained at ambient conditions. The total chromatographic run time was 10 min.

Effect of independent variables on retention time (X):

The effect of the selected independent variables on the retention time of Rivaroxaban was evaluated using the experimental design generated by Design-Expert® software. The response surface linear model was found to be statistically significant, with an F-value of 1494.03 and a *p*-value of less than 0.005. The coefficient of determination ((R²)) was 0.9980, indicating that the model explained 99.80% of the variability in retention time. The coefficient of variation (% CV) and adjusted (R²) were 0.80% and 0.9973, respectively, demonstrating good model fit and low experimental variability. According to the software output, the probability of obtaining a model F-value of this magnitude due to random noise was approximately 0.01%.

The model for response X (Retention time) is as follows:

$$\text{Retention Time} = +6.58 - 0.98 \times A - 6.068E-003 \times B$$

Fig.3 (b) shows a graphical representation of pH of Aqueous Phase (B) and amount of ACN (A), while flow rate (C) is maintained constant at its optimum of 1.10 mL min⁻¹. Change in pH of Aqueous Phase showed slightly change in retention time (X), also increase in amount of Acetonitrile showed decreases the retention time.

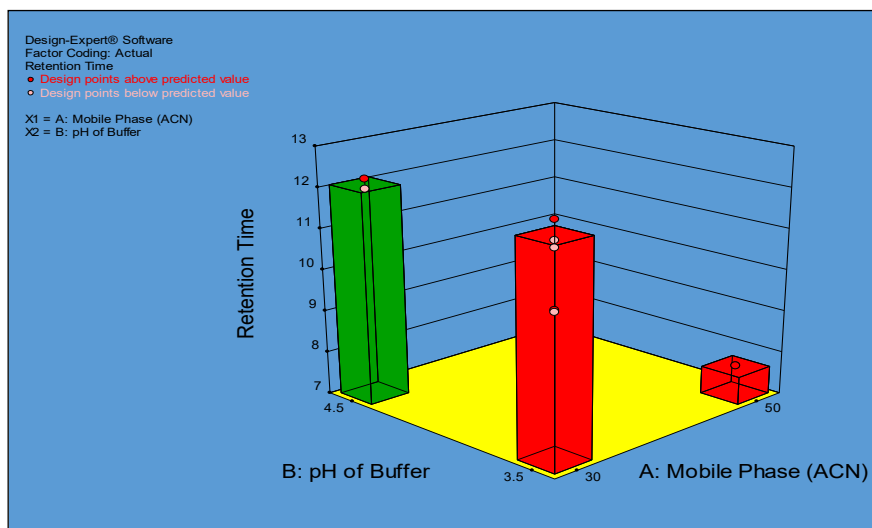


Figure 2: Three-dimensional plot for retention time as a function of pH of Aqueous and amount of acetonitrile. Constant factor (flow rate- 1.10 mL min⁻¹)

Fit summary: Linear model was suggested by the software.

ANOVA: ANOVA of developed Optimal model for retention time (Y₁).

Values of "Prob > F" (p- value) less than 0.0500 indicate model terms are significant. In this case A and B are significant model terms.

Table 3 Significance of *p* value on model terms of retention time

Model terms	<i>p</i> value	Effect of factor	Remarks
A	0.0001	2961	Significant
B	0.7486	0.11	Insignificant
Overall model	0.0001		Significant

Effect of independent variables on tailing factor (Y):

After applying experimental design, suggested Response Surface Linear Model was found to be significant with model F value of 7.73, *p* value less than 0.005 and R² value of 0.8226. There is only a 0.52% chance that a "Model F-Value" this large could occur due to noise. Values of % C.V. and adjusted R² were 7.62 and 0.7161 respectively. The model for response

Y (Tailing factor) is as follows:

$$\text{Asymmetric Factor} = +1.20 - 0.11 * A - 0.038 * B - 0.090 * A * B$$

Fig.6.(b) shows a graphical representation of pH of Aqueous (B) and amount of ACN (A), while flow rate (C) is maintained constant at its optimum of 1.10 mL min⁻¹. A Increases in pH of buffer decrease the tailing factor, it is synergistic effect on response (Y) while increases in amount of Acetonitrile showed decreases in the asymmetry.

Fit summary: Response Surface Linear Model was suggested by the software.

ANOVA : ANOVA of developed Optimal model for tailing factor (Y_2).

Values of "Prob > F" (p- value) less than 0.0500 indicate model terms are significant. In this case B is significant model terms.

Table 4 Significance of p value on model terms of tailing factor

Model terms	p value	Effect of factor	Remarks
A	0.0167	12.48	Significant
B	0.2820	1.45	Significant
Overall model	0.0252	-	Significant

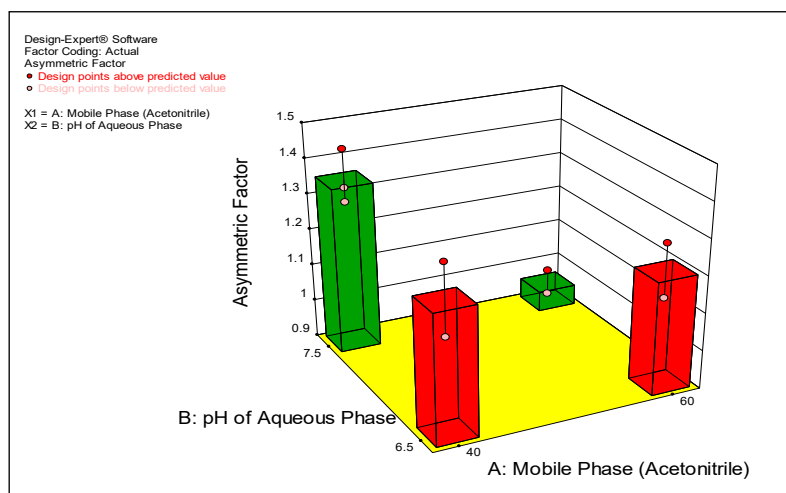


Figure 3: Three-dimensional plot for tailing factor as a function of pH of Aqueous and amount of ACN. Constant factor (flow rate- 1.10 mL min⁻¹)

Effect of independent variables on theoretical plates (Z):

After applying experimental design, suggested Response Surface Linear Model was found to be significant with model F value of 2.66, p value less than 0.005 and R^2 value of 0.6147. There is only a 15.96% chance that a "Model F-Value" this large could occur due to noise. Values of % C.V. and adjusted R^2 were 28.48 and 0.3835 respectively. The model for response Z (theoretical plates) is as follows:

$$\text{Theoretical Plates} = +19790.71 + 4823.54 * A + 2275.71 * B + 857.54 * A * B$$

Fig.9.(b) shows a graphical representation of amount of Acetonitrile (A) and pH of buffer (B), while flow rate (C) is maintained constant at its optimum value 1 mL min⁻¹. A decrease in pH of Aqueous Phase do not showed a significant effect on number of theoretical plates (Z), while Increases in amount of ACN showed increases response.

Fit summary: Linear model was suggested by the software

ANOVA: ANOVA of developed CCD model for theoretical plates (Y_3).

Values of "Prob > F" (p- value) less than 0.0500 indicate model terms are significant. In this case A value is significant model terms.

Table 5 Significance of p value on model terms of theoretical plates

Model terms	p value	Effect of factor	Remarks
A	0.0496	6.64	Significant
B	0.2782	1.48	Insignificant
Overall model	0.1596	-	Insignificant

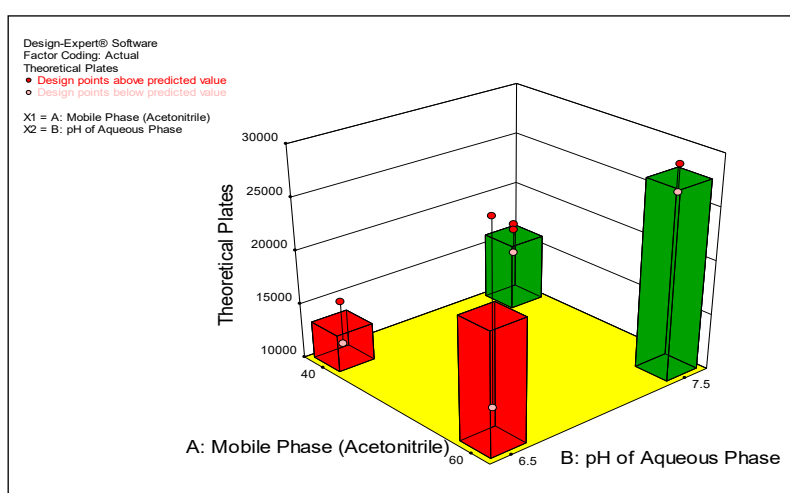


Figure 4: Three-dimensional plot for theoretical plates as a function of pH of Aqueous and amount of ACN. Constant factor (flow rate- 1.10 mL min⁻¹)

5. Result and Discussion

Method Validation:

The developed chromatographic method was validated using ICH guidelines. Validation parameters performed include linearity, limit of detection and quantitation, precision, accuracy, robustness and repeatability as per ICH guidelines

Linearity:

The linearity of this method was determined at ranging from 2-12 µg/ml for Rivaroxaban at 250nm using acetonitrile as solvent. The method for estimation of Rivaroxaban was found to be linear in the range of concentrations 2-12 µg/ml with regression equation were found to be $y = 466803.29x + 13333$ and the correlation coefficient is 0.996. Linearity data shown in Table 6.

Table 6: Linearity Result of Rivaroxaban

Linearity		
Sr. No	Concentration (µg/mL)	Peak Area
1	2	932178
2	4	1864356

3	6	2746534
4	8	3728712
5	10	4660890
6	12	5593068
Slope		466803.29
Standard Error		22625.31

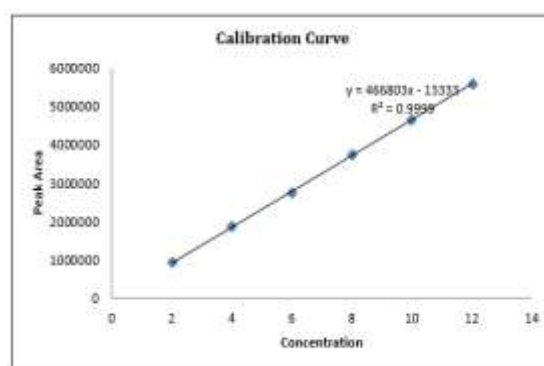


Figure 5: Calibration Curve of Rivaroxaban

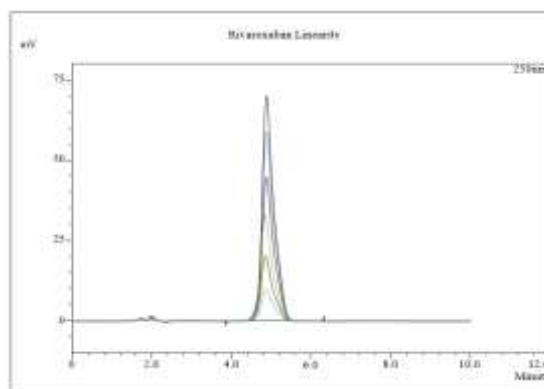


Figure 6: Chromatogram for Overlay

Table 7: Characteristic Parameters of Rivaroxaban for the proposed HPLC method.

Sr.no.	Parameter	Results
1	Calibration range (µg/ml)	2 - 12
2	Regression equation (y*)	$y = 466803.29x - 13333$
3	Slope (b)	466803.29
4	Intercept (a)	13333
5	Correlation coefficient(r2)	0.9999

System Suitability:

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (Rt), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the drug at a concentration of 2 µg/ml. The results which are given in Table 8. Were within acceptable limits.

Table 8: System Suitability Studies of Rivaroxaban By HPLC Method.

System Suitability Parameter		
Retention time (min)	Concentration (µg/mL)	5.587
Peak area	2	932178
Theoretical plates		27745
Asymmetric Factor		0.957

Specificity:

Chromatogram of blank was taken as shown in Figure 7, 8 & 9 Chromatogram of Rivaroxaban showed peak at a retention time of 5.567 min. The mobile phase designed for the method resolved the drug very efficiently. The Retention time of Rivaroxaban sample was 5.564 min. The wavelength 250nm was selected for detection because; it resulted in better detection sensitivity for the drug. The peak for Rivaroxaban from the injection was Rivaroxaban.

Table 9: Specificity of Rivaroxaban by HPLC Method.

Specificity				
Sample	Label Claim (mg)	Amount Found	Recovery	Retention Time
Tablet	15	14.91	99.4	5.587

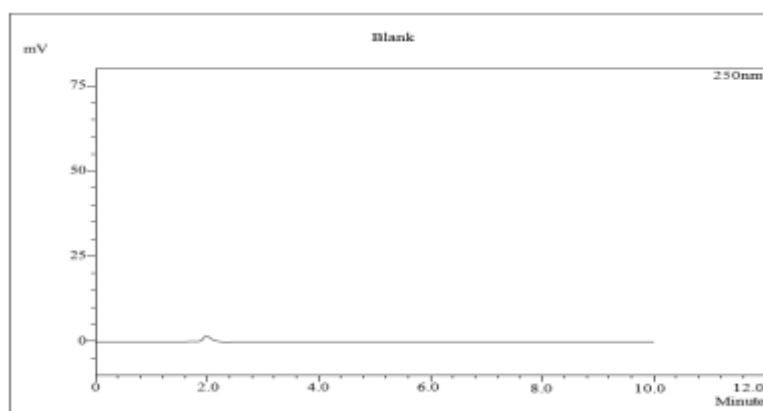


Figure 7: Chromatogram of Blank

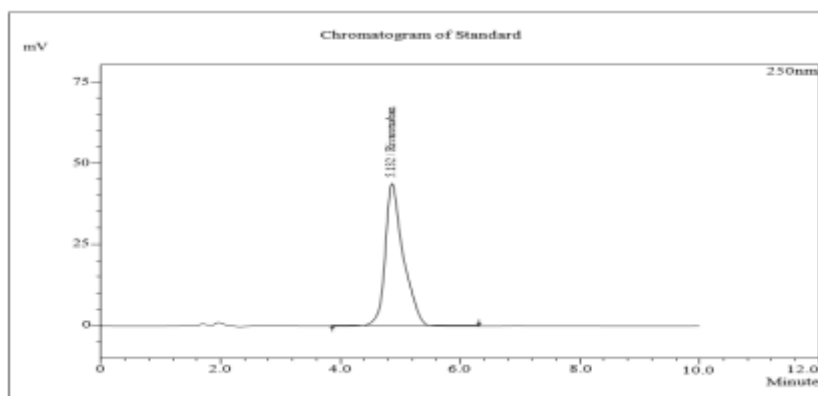


Figure 8: A typical chromatogram of Rivaroxaban Standard [Concentration 8 ug/ml]

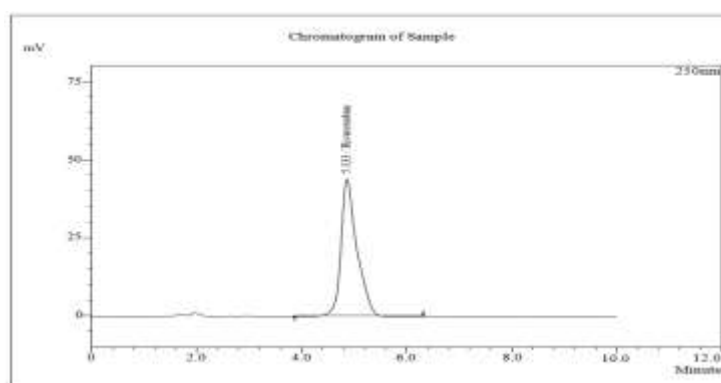


Figure 9: A typical chromatogram of Rivaroxaban Sample [Concentration 8 ug/ml]

Precision:

Precision studies were carried out to ascertain the reproducibility of the proposed method. This study divided into repeatability, intraday and interday.

The injection repeatability (System Precision) was assessed by using six injections of the standard solution of Rivaroxaban and the % RSD of the replicate injections was calculated. In addition, to demonstrate the precision of method (Method Precision), six samples from the same batch of formulation were analyzed individually and the assay content of each sample was estimated. The average for the six determinations was calculated along with the % RSD for the replicate determinations. Both the system precision and method precision were subjected for interday and intra-day variations as reported in Table 10.

Table 10: Precision.

Precision			
Sr. No	Concentration (µg/mL)	Intraday	Interday
1	8	3753188.5	3741551
2	8	3778213	3782461.333
3	8	3604826	3867874

4	8	3760193	3647504
5	8	3791948	3728414
6	8	3765399.25	3694135
Average		3742294.6	3743656.6
Standard Deviation		62750.91	69366.48
RSD%		1.6768	1.853

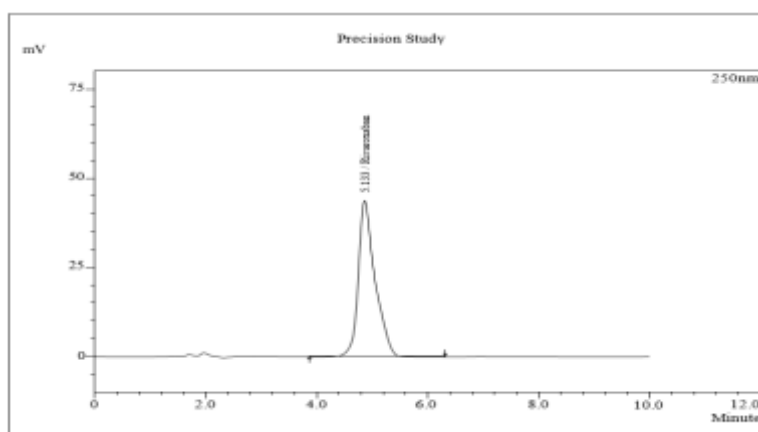


Figure 10: A typical chromatogram of Precision Study

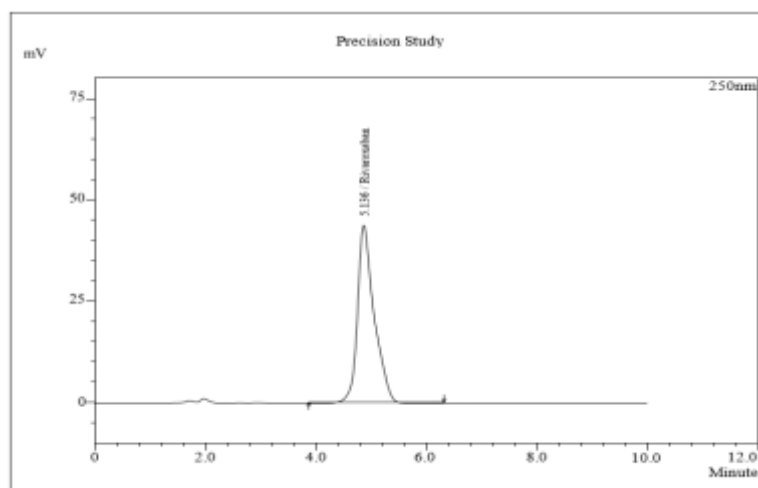


Figure 11: A typical chromatogram of Precision Study

Accuracy:

The accuracy for the analytical method for Rivaroxaban was determined at 80%, 100% and 120% levels of standard solution and results were expressed in terms of % recoveries. Standard deviation and % RSD were calculated. The results were tabulated in (Table 11).

Table 11: Accuracy of Rivaroxaban at 210 nm.

Recovery				
Sr. No	Amount of Sample	Amount of Drug Added	Amount of Drug Recovered	Recovery %
	(µg/ml)	(µg/ml)	(µg/ml)	
1	8	4	3.989	99.73
2	8	8	7.99	99.89
3	8	12	12.01	100.08

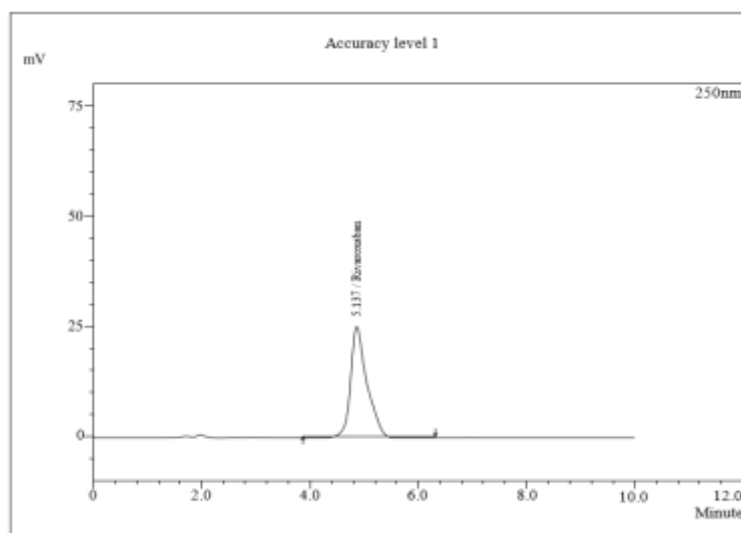


Figure 12: A typical chromatogram of Accuracy Level 1

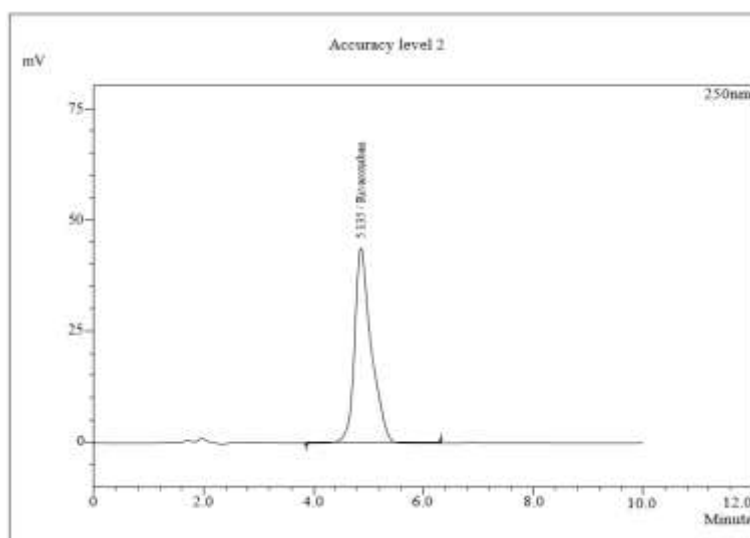


Figure 13: A typical chromatogram of Accuracy Level 2

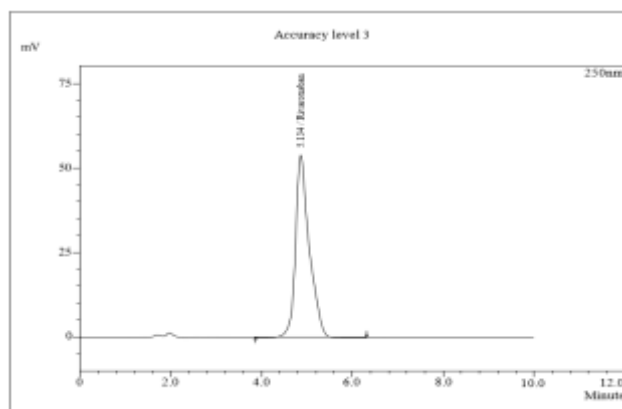


Figure 14: A typical chromatogram of Accuracy Level 3

Robustness:

Robustness is a measure of capacity of a method to remain unaffected by small, but deliberate variations in the method conditions, and is indications of the reliability of the method. A method is robust, if it is unaffected by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at three different levels and retention time and chromatographic response were evaluated. One factor at a time was changed to study the effect. Variation of wavelength, mobile phase, pH and flow rate by 1.0 ml/min (1.1 and 1.2 ml/min) had no significant effect on the retention time and chromatographic response of the 8 µg/ml solution, indicating that the method was robust. The results are shown in Table No 12.

Table 12: Robustness Of At Rivaroxaban

Robustness						
Sr. No		Parameter		Response	Parameter	Response
Methanol : Water			Retention Time (min)	Detection Wavelength	Peak Area	
(V/V)				(nm)		
1	59	41	5.49	248	3670585	
2	60	40	5.587	250	3728900	
3	61	39	5.686	252	3762164	
Average			5.588	Average	3720550	
Standard Deviation			0.080	Standard Deviation	37850.36	
RSD%			1.432	RSD%	1.017	
Flow Rate			Retention Time (min)	pH of Buffer	Peak Area	
(mL/min)				(mmol/L)		
1	1		5.718	7.3	3756972	
2	1.1		5.587	7.5	3729073	

3	1.2	5.473	7.7	3653452
Average		5.593	Average	3713166
Standard Deviation		0.1001	Standard Deviation	43733.13
RSD%		1.790	RSD%	1.1778

6. Discussion

The present study focused on the development and validation of a reversed-phase high-performance liquid chromatography (RP-HPLC) method for the estimation of Rivaroxaban in bulk drug and tablet dosage forms. The developed method employed a Waters XBridge C18 analytical column, an acetonitrile–water mobile phase, and UV detection at 250 nm. The method was evaluated for linearity, system suitability, specificity, precision, accuracy, and robustness to assess its suitability for quantitative analysis.

The linearity of the proposed method was evaluated over a concentration range of 2–12 µg/mL. The calibration data demonstrated an increase in peak area with increasing Rivaroxaban concentration, indicating a concentration-dependent detector response. The reported regression equation was ($y = 466803.29x + 13333$), with a slope of 466803.29 and an intercept of 13333. The reported correlation coefficient was 0.996 in the initial results, whereas the characteristic-parameter table reported an (R^2) value of 0.9999.

These results suggest a strong linear relationship between Rivaroxaban concentration and chromatographic response over the investigated range. However, the difference between the reported correlation statistics should be resolved by recalculating the regression using the original calibration data. The slope indicates the change in peak area corresponding to a unit increase in concentration, while the intercept represents the predicted response at zero concentration. Confirmation of the regression statistics is essential to establish the reliability of the calibration model.

System suitability was evaluated using retention time, theoretical plate number, and peak asymmetry. The reported retention time was 5.587 min, the theoretical plate number was 27,745, and the asymmetry factor was 0.957. The retention time indicates consistent chromatographic elution under the selected conditions, while the theoretical plate number suggests satisfactory column efficiency. The asymmetry factor, being close to 1, indicates a relatively symmetrical chromatographic peak.

Overall, the reported parameters suggest that the chromatographic system provided suitable separation characteristics for Rivaroxaban under the selected operating conditions. Nevertheless, the table should be checked to confirm whether the reported values represent the mean of six replicate injections and whether the applicable system-suitability acceptance criteria were met.

Specificity was investigated by examining the chromatograms of the blank, Rivaroxaban standard, and tablet sample. The standard and sample chromatograms showed Rivaroxaban peaks at approximately 5.56–5.59 min. The close agreement between the reported retention times of the standard and tablet sample supports the identification of Rivaroxaban in the formulation. The blank chromatogram was also included to assess the presence of interfering signals at the analyte retention time.

The tablet analysis yielded 14.91 mg of Rivaroxaban against a labelled claim of 15 mg, corresponding to 99.4% of the labelled amount. This result indicates satisfactory agreement between the measured drug content and the declared tablet strength. However, the absence of a co-eluting peak in the blank alone does not establish complete specificity in a tablet matrix. Further evidence, such as placebo chromatograms or peak-purity

assessment, would strengthen the demonstration that excipients do not interfere with Rivaroxaban quantification.

The precision of the method was evaluated using intraday and interday measurements at a Rivaroxaban concentration of 8 $\mu\text{g/mL}$. The reported mean peak areas were 3,742,294.6 and 3,743,656.6 for intraday and interday precision, respectively. The corresponding standard deviations were 62,750.91 and 69,366.48, with percentage relative standard deviations (%RSD) of 1.6768% and 1.853%, respectively.

The relatively low %RSD values indicate limited variability in the measured chromatographic responses across the reported measurements. The similar mean peak areas obtained for intraday and interday analyses also suggest that the method may provide consistent responses under the investigated conditions. These findings support the potential suitability of the method for routine quantitative analysis. Nevertheless, the reported precision data should be checked against the original chromatograms and calculations before a definitive claim of compliance with the applicable acceptance criteria is made.

Accuracy was assessed at three concentration levels corresponding to 80%, 100%, and 120% of the nominal test concentration. The reported recoveries were 99.73%, 99.89%, and 100.08%, respectively. These values are close to 100%, indicating good agreement between the amounts of Rivaroxaban added and recovered under the experimental conditions.

The narrow range of reported recoveries suggests that the proposed method may provide reliable quantitative measurements across the evaluated levels. However, the table should clearly distinguish the amount of drug added from the total amount recovered, particularly because the stated sample concentration was 8 $\mu\text{g/mL}$. The recovery calculations should be verified using the actual sample and spiked concentrations to ensure that the reported percentages accurately represent the accuracy of the analytical procedure.

Robustness was evaluated by introducing small, deliberate variations in mobile-phase composition, detection wavelength, flow rate, and aqueous-phase pH. The reported retention times ranged from 5.49 to 5.686 min for changes in mobile-phase composition, while the peak areas varied with changes in detection wavelength. Flow-rate variations between 1.0 and 1.2 mL/min resulted in retention times ranging from 5.473 to 5.718 min. Changes in aqueous-phase pH from 7.3 to 7.7 were associated with differences in the recorded peak areas.

The reported %RSD values ranged from 1.017% to 1.790% across the summarized robustness comparisons. These results suggest that the method maintained a measurable chromatographic response under the investigated variations. However, changes in retention time and peak area were observed, indicating that the method was not completely unaffected by changes in operating conditions. Robustness should therefore be assessed using predefined acceptance criteria and the effect of each variation on the critical analytical responses.

It is also necessary to verify the mobile-phase composition stated in the robustness table, which is labelled methanol–water, against the optimized acetonitrile–water mobile phase described elsewhere in the method. If methanol was used as a deliberate change in solvent identity, this experiment should be reported separately from minor variations in mobile-phase composition. In addition, the table should clearly identify the units and experimental levels for pH, wavelength, and flow rate.

The reported findings indicate that the developed RP-HPLC procedure has potential for the quantitative estimation of Rivaroxaban in bulk drug and tablet dosage forms. The observed retention time, theoretical plate number, peak asymmetry, recovery values, and precision results support further consideration of the method for routine analytical applications. Nevertheless, discrepancies in the linearity statistics, the regression equation, the robustness conditions, and some table entries must be resolved before the method can be considered fully validated. Additional reporting of the limit of detection (LOD), limit of quantitation (LOQ), and other required validation results would provide a more complete assessment of its analytical performance.

7. Conclusion

A reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed for the quantitative estimation of Rivaroxaban in bulk drug and tablet dosage forms using a Waters XBridge C18 column and UV detection at 250 nm. The optimized chromatographic conditions included an acetonitrile–water mobile phase (60:40, v/v), an aqueous-phase pH of 7.5, a flow rate of 1.10 mL/min, an injection volume of 10 μ L, and a total run time of 10 min. Under these conditions, Rivaroxaban exhibited a retention time of approximately 5.59 min.

The reported validation results demonstrated satisfactory drug recovery, with values ranging from 99.73% to 100.08%, and relatively low variability in intraday and interday peak-area measurements. The reported theoretical plate number of 27,745 and asymmetry factor of 0.957 further indicated favorable chromatographic efficiency and peak shape. Analysis of the marketed tablet formulation yielded 14.91 mg of Rivaroxaban against a labelled claim of 15 mg, corresponding to 99.4% of the labelled amount.

Although these findings indicate the potential applicability of the proposed method for routine quality-control analysis, the discrepancies in the reported linearity statistics and the robustness data should be resolved. The LOD and LOQ values and the complete validation results should also be reported and evaluated against predefined acceptance criteria. Following verification of the experimental data and confirmation of the applicable ICH validation requirements, the method may be considered a potential analytical procedure for the routine estimation of Rivaroxaban in pharmaceutical formulations.

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