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(RESEARCH ARTICLE)

Quality by Design (QbD)–Based RP-HPLC Method Development and Validation for Apixaban

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Abstract

A robust reverse phase high-performance liquid chromatographic (RP-HPLC) method for the quantitative determination of apixaban was developed using a Quality by Design (QbD) approach. The Analytical Target Profile (ATP) was defined to achieve adequate resolution, acceptable retention time, and peak symmetry. Critical Method Parameters (CMPs) were identified and optimized using Design of Experiments (DoE) and statistical evaluation through Analysis of Variance (ANOVA). The optimized method was validated according to ICH Q2 guidelines and demonstrated suitable linearity, precision, accuracy, robustness, and stability-indicating capability. The developed method is suitable for routine quality control analysis of apixaban in bulk and pharmaceutical dosage forms.

Keywords: Apixaban; Quality by Design; RP-HPLC; DoE; ANOVA; Method Validation

1. Introduction

High-performance liquid chromatography (HPLC) is widely employed in pharmaceutical analysis due to its accuracy, sensitivity, and reproducibility. Traditional method development approaches are often based on trial-and-error experimentation, which may result in poor robustness and method variability. The Quality by Design (QbD) approach introduces a systematic, science- and risk-based methodology that enhances method understanding and ,lifecycle performance.

Apixaban is a selective direct factor Xa inhibitor used for the prevention and treatment of thromboembolic disorders. Given its therapeutic importance, a reliable and robust analytical method is essential for its quality control. The present study focuses on the development and validation of a QbD-based RP-HPLC method for apixaban.

2. Materials and Methods

Apixaban working standard and the marketed formulation (Eliquis Tablets IP) were procured from reliable pharmaceutical sources and used for method development and validation studies. The working standard of Apixaban was obtained from AbMole BioScience, while the marketed formulation was sourced from Emcure Pharmaceuticals Ltd. All chemicals and reagents employed in the study were of analytical reagent (AR) or HPLC grade to ensure analytical accuracy and reproducibility. HPLC-grade methanol and water were used for mobile phase preparation, whereas hydrochloric acid, sodium hydroxide, and hydrogen peroxide were utilized for forced degradation studies under acidic, alkaline, and oxidative conditions, respectively. Chromatographic analysis was carried out using a high-performance liquid chromatography (HPLC) system equipped with a UV detector. The instrumentation included an HPLC system with UV detection, a Cosmosil C18 analytical column (250 × 4.6 mm, 5 µm), analytical balance, sonicator, and digital pH meter. Detailed chromatographic separation and validation were performed using an Agilent 1260 Infinity II HPLC system

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fitted with an ultraviolet detector, PU-2080 Plus pump, Inertsil ID column (4.6 × 250 mm), and Open Lab EZ Chrome software for data acquisition and processing. The Analytical Target Profile (ATP) was defined to ensure accurate quantification with acceptable system suitability parameters. Risk assessment was performed to identify CMPs such as mobile phase composition, flow rate, and detection wavelength.

2.1. Quality by Design and Experimental Design

Clinical studies have established Apixaban as an effective oral anticoagulant at low dose levels, necessitating the development of sensitive and reliable analytical methods for its quantitative determination. A comprehensive review of the literature revealed that Apixaban has been analyzed using various analytical techniques such as UV spectrophotometry, high-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC-MS), and high-performance thin-layer chromatography (HPTLC) for estimation from bulk drug, pharmaceutical dosage forms, and biological matrices. However, most of the reported chromatographic methods were developed using conventional trial-and-error approaches and lacked systematic control over method variability and robustness. Furthermore, limited studies have focused on the application of Analytical Quality by Design (AQbD) principles for the development of RP-HPLC methods for Apixaban. In view of current regulatory expectations emphasizing risk-based analytical development, the present study was undertaken to develop and validate a QbD-based RP-HPLC method for the quantitative estimation of Apixaban, with an objective of minimizing analytical variability and enhancing method robustness throughout the analytical lifecycle.

2.2. Materials and Methods Instruments and Chemicals

Chromatographic analysis was performed using a high-performance liquid chromatography (HPLC) system equipped with a UV detector and capable of isocratic operation. Data acquisition and chromatogram processing were carried out using appropriate chromatographic software. Separation was achieved on a C18 reverse-phase analytical column with dimensions of 250 mm × 4.6 mm and particle size of 5 µm. An analytical balance with a sensitivity of 0.1 mg was used for accurate weighing of samples, and a digital pH meter was employed for pH adjustment where required. Apixaban working standard was obtained from a reputed pharmaceutical source, while HPLC-grade methanol and water were used for mobile phase preparation. Hydrochloric acid, sodium hydroxide, and hydrogen peroxide of analytical reagent grade were used for forced degradation studies. All chemicals and reagents used were of analytical or HPLC grade.

2.3. Factor Screening Studies

Based on preliminary literature review and risk assessment, critical method parameters (CMPs) such as mobile phase composition, flow rate, and detection wavelength were identified as potential factors influencing chromatographic performance. These parameters were evaluated to study their individual and combined effects on critical quality attributes (CQAs), including retention time, peak symmetry, and theoretical plate count. Factor screening studies were conducted using a structured Design of Experiments (DoE)approach to establish a scientific understanding of method variability and parameter interaction.

2.4. Preparation of Standard Solution

An accurately weighed quantity of Apixaban working standard was transferred into a volumetric flask, dissolved in methanol with sonication, and diluted to the required volume using the same solvent to obtain a standard stock solution. Aliquots of this stock solution were further diluted with methanol to prepare working standard solutions of appropriate concentrations for method development and validation studies.

2.5. Experimental Design for Method Development

The analytical wavelength for method development was selected by scanning the Apixaban working solution over a wavelength range of 200–400 nm using a UV spectrophotometer. A factorial experimental design was employed to study the combined effect of selected CMPs on the defined CQAs. Design-Expert® software was used to generate the experimental design matrix and analyze the responses obtained from each experimental run. All chromatographic experiments were performed using a fixed concentration of Apixaban, and the responses were evaluated in terms of retention time, peak symmetry, and column efficiency.

2.6. Optimization and Data Analysis

2.6.1. Optimized Chromatographic Conditions

Based on the results obtained from Analytical Quality by Design (AQbD)–driven optimization, the chromatographic conditions were finalized using numerical desirability analysis. The optimized method employed a mobile phase

consisting of methanol and water in a proportion of approximately 70:30 (v/v), delivered at a flow rate of 0.8 mL/min. Chromatographic separation was achieved on a C18 reverse-phase column (250 × 4.6 mm, 5 µm particle size), and detection was carried out using a UV detector at a wavelength of 223 nm. Under these conditions, Apixaban exhibited satisfactory chromatographic performance with appropriate retention, good peak symmetry, and high column efficiency.

The optimized conditions were selected to ensure robustness, reproducibility, and compliance with regulatory expectations for routine quality control analysis.

2.6.2. Simulation of Optimized Chromatographic Conditions

The experimentally obtained chromatogram closely matched the predicted responses, demonstrating a retention time of approximately 5.0 min with a well-resolved and symmetric peak. The peak area and theoretical plate count confirmed adequate sensitivity and column efficiency, while the asymmetry factor remained within acceptable limits. This close agreement between predicted and observed chromatographic behavior validates the robustness of the developed AQbD model and confirms the reliability of the selected optimized conditions.

Table 1 Optimized Conditions as per Annova

Run	Factor-1 Methanol: Water	Factor-1 Flow Rate	Response-1 Retention Time	Response-2 Tailing Factor	Response Theoretical Plate
1.	60:40	1.2	3.709	1.069	5673
2.	80:20	1.0	4.437	1.066	7306
3.	70:30	0.8	4.029	1.22	4879
4.	70:30	1.0	4.276	1.096	6392
5.	80:20	1.0	4.448	1.066	5879

3. Results and Discussion

Optimization studies revealed that mobile phase composition and flow rate had a significant impact on chromatographic performance. ANOVA results indicated statistically significant models with acceptable R^2 values and non-significant lack-of-fit, confirming model adequacy.

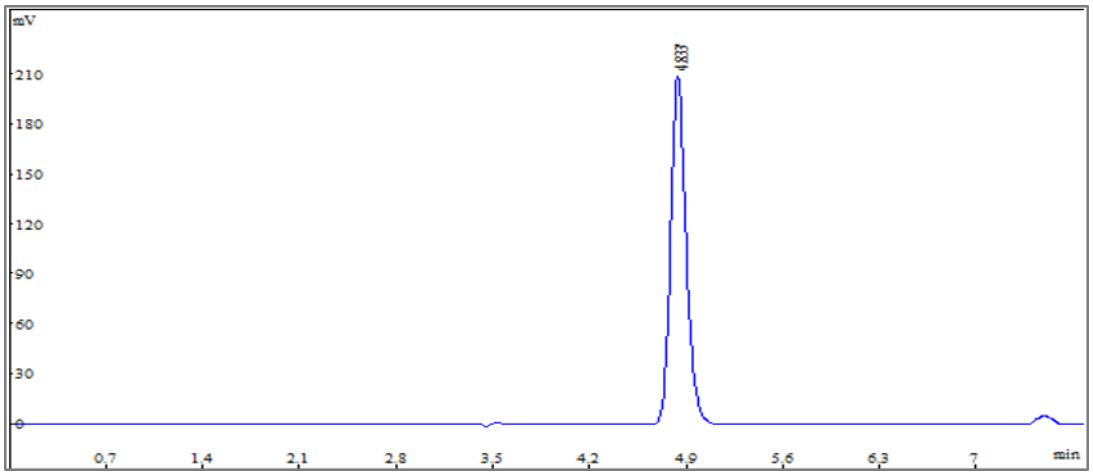

Figure 1 Optimized chromatogram of Apixaban

3.1. System suitability

testing is a critical component of chromatographic method development and validation, as it ensures that the analytical system is capable of producing reliable and reproducible results before sample analysis. System suitability parameters were evaluated using a standard solution of Apixaban at a concentration of 50 µg/mL,

The results demonstrated that the percentage relative standard deviation (%RSD) for both retention time and peak area was within 1%, indicating excellent system precision and column efficiency. The theoretical plate count was found to be greater than 7000, confirming adequate column performance. The tailing factor was close to unity, reflecting good peak symmetry, while the retention time remained consistent throughout the analysis. All evaluated system suitability parameters complied with the recommended acceptance criteria, confirming that the chromatographic system was suitable for method validation and routine analysis.

Table 2 System suitability parameters for Apixaban

Parameter	Concentration (50 µg/mL)
Theoretical plates	7306
Tailing factor	1.066
Retention time (min)	4.437
Peak area	668,724

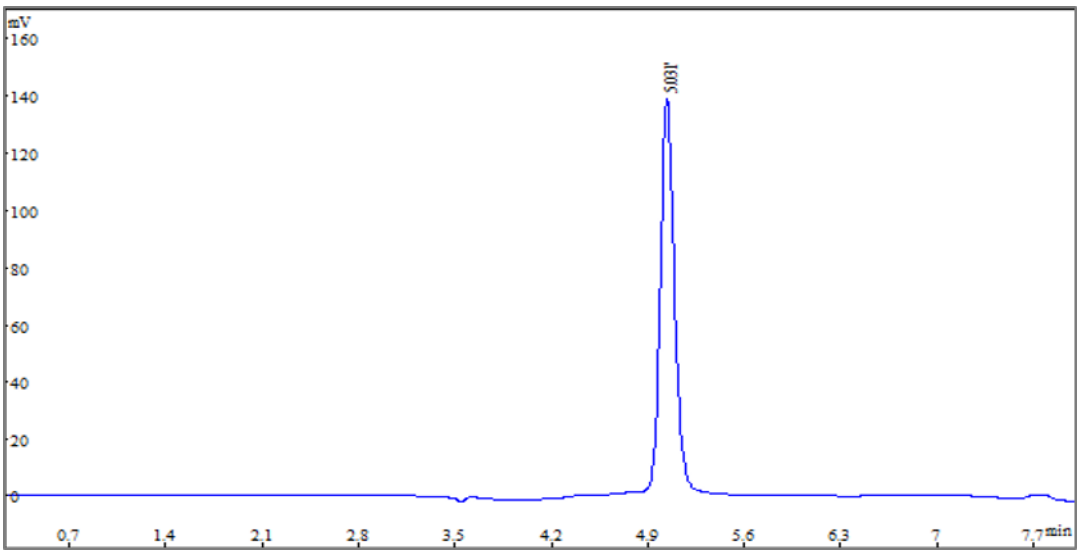

Figure 2 System suitability chromatogram of Apixaban

3.2. Linearity:

of the developed RP-HPLC method for Apixaban was evaluated by analyzing standard solutions at different concentration levels. Calibration curves were constructed by plotting peak area against the corresponding concentration of Apixaban and subjected to least-squares linear regression analysis to determine the calibration equation and correlation coefficient.

The method exhibited a linear response over the concentration range of 10–50 µg/mL. A strong linear relationship between concentration and peak area was observed, with a correlation coefficient (r^2 = 0.9991), indicating excellent linearity of the method. The regression equation obtained for Apixaban was y = 35152x – 38348, confirming proportional detector response across the studied concentration range. The results of the calibration curve data are presented in Table 25.

Table 3 Calibration curve data for Apixaban by RP-HPLC method

Sr. No.	Concentration (µg/mL)	Peak Area
1	10	301,630
2	20	665,331
3	30	1,028,466
4	40	1,387,538
5	50	1,698,142

The optical and regression characteristics of Apixaban obtained by the HPLC method are summarized

Table 4 Optical and regression characteristics of Apixaban

Sr. No.	Parameter	HPLC Method
1	Detection wavelength (λmax)	223 nm
2	Linearity range (µg/mL)	10–50
3	Regression equation	y = 35152x – 38348
4	Slope (m)	35152
5	Intercept (c)	38348
6	Correlation coefficient (r^2)	0.9991
7	Limit of Detection (LOD)	0.13 µg/mL
8	Limit of Quantification (LOQ)	0.42 µg/mL

The high correlation coefficient and low values of LOD and LOQ demonstrate that the developed method is sensitive and suitable for quantitative estimation of Apixaban at low concentration levels.

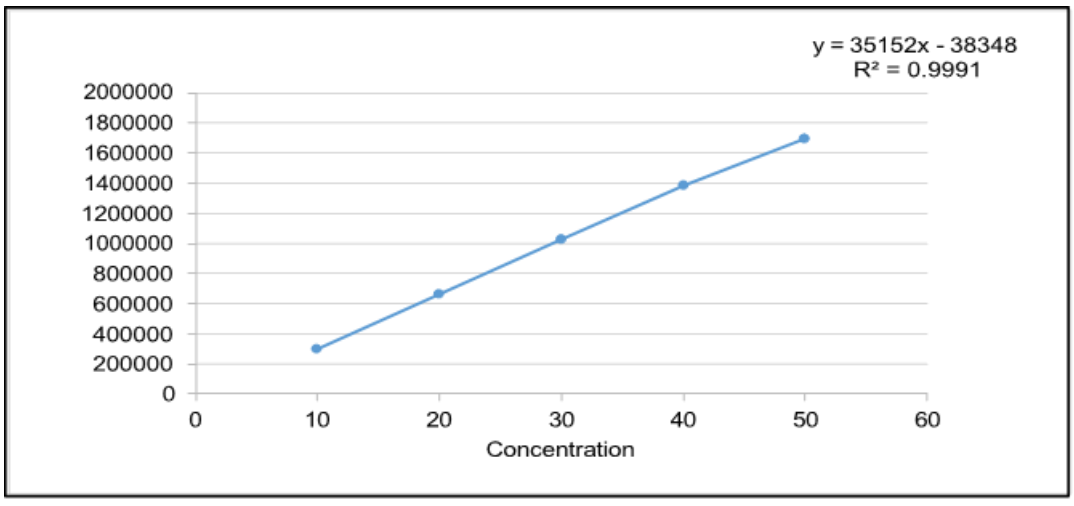

Figure 3 Calibration curve of Apixaban

3.3. Accuracy

Accuracy of the developed RP-HPLC method was evaluated by recovery studies at three concentration levels corresponding to 50%, 100%, and 150% of the target concentration. The accuracy results are summarized in Table 27.

The mean percentage recovery of Apixaban at all three levels was found to be within the acceptable range of 98–102%, indicating the accuracy of the proposed method.

The %RSD values for all recovery levels were below 2%, confirming that the method is accurate and free from interference by excipients present in the formulation.

Table 5 Recovery study of Apixaban by RP-HPLC method

Level	Area of Standard	Area of Sample	% Recovery	Conc. Taken (ppm)	Conc. Found (ppm)
50%	1,028,466	1,021,050	99.28	30	29.78
100%	1,387,538	1,384,232	99.76	40	39.90

Table 6 Statistical evaluation of accuracy

Level	SD	%RSD
50%	76.17	0.094
100%	88.32	0.068
150%	15.50	0.01

3.4. Precision

Precision of the developed method was evaluated in terms of intra-day and inter-day precision at a concentration of 30 µg/mL. The results, expressed as %RSD, are presented in Table 29. The %RSD values for both intra-day and inter-day precision were found to be well below 2%, demonstrating excellent repeatability and intermediate precision of the method.

Table 7 Intra-day and inter-day precision data of Apixaban

Precision Type	Conc. (µg/mL)	Mean Area	SD	%RSD
Intra-day	30	1,027,239	3002.80	0.292
Intra-day	30	1,023,108	2329.15	0.228
Inter-day	30	1,021,826	4261.65	0.417
Inter-day	30	1,027,239	3002.80	0.292

3.5. Repeatability

Repeatability was assessed by analyzing Apixaban at three concentration levels under the same operating conditions over a short time interval. The results summarized in Table 30 demonstrate consistent recovery values with %RSD well below the acceptance limit, confirming the repeatability of the method.

Table 8 Repeatability study of Apixaban

Conc. (µg/mL)	Mean Found	% Recovery
30	29.2	98.5
40	39.2	98.6
50	49.8	99.2

Mean recovery: 98.6%

3.6. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The sensitivity of the RP-HPLC method was evaluated by determining the limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ values were found to be 0.13 µg/mL and 0.39 µg/mL, respectively, indicating that the method is sufficiently sensitive for the quantification of Apixaban.

Table 9 LOD and LOQ values of Apixaban

Parameter	Value
LOD	0.13 µg/mL
LOQ	0.39 µg/mL

3.7. Robustness

Robustness of the developed RP-HPLC method was evaluated by introducing deliberate variations in chromatographic conditions, including changes in pH and detection wavelength. The results of the robustness study are presented in Table 32. The %RSD values obtained under varied conditions were less than 2%, indicating that minor deliberate changes did not significantly affect method performance.

Table 10 Robustness study of Apixaban

Change in pH

Conc. (µg/mL)	Mean Area	SD	%RSD
20	663,496	1924.51	0.29

Change in wavelength

Conc. (µg/mL)	Mean Area	SD	%RSD
20	665,043	3415.60	0.51

3.8. Ruggedness

Ruggedness of the method was evaluated by performing the analysis using different analysts under similar experimental conditions. The %RSD values obtained were below 2%, confirming the ruggedness and reproducibility of the method.

Table 11 Ruggedness study of Apixaban

Analyst	Conc. (µg/mL)	Mean Peak Area	SD	%RSD
Analyst 1	30	1,027,239	0.292	0.127
Analyst 2	50	1,694,141	4165.42	0.128

3.9. Forced Degradation Studies

Forced degradation studies were conducted on Apixaban at a concentration of 50 µg/mL under acidic, alkaline, oxidative, photolytic, and thermal stress conditions. The results summarized in Table 34 indicate that Apixaban was stable under the applied stress conditions, and no significant degradation was observed. The developed RP-HPLC method effectively separated the analyte from potential degradation products, confirming its stability-indicating capability.

Table 12 Forced degradation study of Apixaban

Stress Condition	% Degradation
Acidic	0
Alkaline	0
Oxidative	0
Photolytic	0
Thermal	0

3.10. Analysis of Marketed Formulation

The developed RP-HPLC method was successfully applied to the assay of Apixaban in a marketed tablet formulation (Eliquis). The assay results are presented in Table 35. The percentage assay was found to be within acceptable limits, confirming the suitability of the method for routine quality control analysis.

Table 13 Assay of Apixaban in marketed formulation

Formulation	Label Claim	Observed Amount (mg)	% Assay
Eliquis Tablets	Apixaban Tablets IP 200 mg	4.98	99.99

4. Conclusion

A robust, precise, and stability-indicating RP-HPLC method for the quantitative estimation of Apixaban was successfully developed and validated using an Analytical Quality by Design (AQbD) approach. Systematic risk assessment and Design of Experiments (DoE) enabled the identification and control of critical method parameters, ensuring enhanced method understanding and minimized analytical variability. Statistical evaluation through ANOVA confirmed the significance and adequacy of the developed models, while numerical optimization based on desirability functions facilitated the selection of optimal chromatographic conditions.

The optimized method demonstrated satisfactory system suitability, excellent linearity over the studied concentration range, high accuracy with recovery values within acceptable limits, and precise repeatability and intermediate precision with %RSD values below 2%. The method exhibited adequate sensitivity, as reflected by low LOD and LOQ values, and remained robust and rugged under deliberate variations in chromatographic conditions and analyst changes. Forced degradation studies confirmed that the method is stability-indicating, with effective separation of Apixaban from potential degradation products.

Furthermore, successful application of the method to the assay of a marketed formulation validated its suitability for routine quality control analysis.

Overall, the developed AQbD-based RP-HPLC method fulfills current regulatory expectations and offers a reliable, reproducible, and efficient analytical tool for the quality control and stability assessment of Apixaban in bulk drug and pharmaceutical dosage forms.

Future Scope

The developed AQbD-based RP-HPLC method can be further extended for the simultaneous estimation of Apixaban in combination formulations and for impurity profiling studies. The systematic understanding of critical method parameters established in this study provides a strong foundation for method transfer, lifecycle management, and continuous improvement in accordance with regulatory expectations. Additionally, the method may be adapted for bioanalytical applications or coupled with advanced detection techniques such as LC–MS for enhanced sensitivity and selectivity. Future work may also focus on applying the AQbD framework to stability studies under long-term and accelerated conditions to further strengthen the analytical control strategy.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no competing interests.

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