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Development And Validation Of An Rp-Hplc Method For Quantitative Estimation Of Apixaban In Pharmaceutical Dosage Forms

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ABSTRACT

A simple, precise, and accurate reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated to measure Apixaban (APIX) in pharmaceutical formulations. Chromatographic separation was achieved using a C18 column, a mobile phase made up of acetonitrile and water in a 70:30 (v/v) ratio, a flow rate of 1.0 mL/min, and detection at 280 nm. In compliance with ICH guidelines, the procedure's linearity, precision, accuracy, selectivity, sensitivity, recovery, and robustness were all verified. Over the range of 5.0–40.5 µg/mL, APIX demonstrated strong linearity and a correlation coefficient (R2) of 0.9997. Intra-day and inter-day precision trials verified the approach's dependability, showing accuracy ranging from -1.89% to 2.67% with RSD values below 3%. Forced degradation studies indicated that APIX was stable under acidic and neutral conditions but degraded significantly under alkaline, thermal, and photolytic stress. When the technique was applied to commercially available tablet formulations, the average content was 9.19 ± 0.05 mg per tablet, with recovery rates ranging from 99.05% to 101.67%. The created technique indicates stability and is appropriate for regular quality control examinations of Apixaban in tablet dosage forms.

Keywords: *Apixaban, Method Validation, Stability-Indicating Assay, RP-HPLC, Pharmaceutical Analysis*

1. INTRODUCTION

Apixaban is a common anticoagulant used to prevent and treat thromboembolic conditions like stroke, pulmonary embolism, and deep vein thrombosis in individuals with non-valvular atrial fibrillation. It is a direct, oral, and specific Factor Xa inhibitor. In contrast to warfarin, it has a positive pharmacokinetic profile, which includes a predictable dose-response, a minimal risk of food and medication interactions, and the absence of the need for regular monitoring. Consequently, Apixaban has become a preferred option among novel oral anticoagulants (NOACs) and has gained considerable therapeutic significance. The creation of a reliable, validated analytical technique for its measurement in pharmaceutical formulations is essential for both regulatory and industrial reasons, given its growing therapeutic applications (Byon et al. 2019; Agnelli et al. 2013; Healey et al. 2024).

High-performance liquid chromatography (HPLC) is a widely utilised analytical technique for quantitative drug analysis due to its exceptional precision, reproducibility, and specificity. Among the various modes of HPLC, reverse-phase (RP-HPLC) has emerged as the most suitable method for analyzing drugs like Apixaban, which are moderately hydrophobic. Despite the availability of some reported methods for Apixaban estimation, many are limited by long run times, complex sample

preparation, insufficient sensitivity, or lack of comprehensive validation including forced degradation studies. This emphasizes the need for an improved method that is not only precise and accurate but also rapid, sensitive, and stability-indicating (Ali 2022; Schieppati et al. 2021).

The current study solves these limitations by developing a simple, reproducible RP-HPLC method that is perfect for the quantitative assessment of Apixaban in tablet dose forms as well as bulk medication. The technique was created to provide quick analysis with high adherence to ICH Q2 (R1) validation parameters, little excipient interference, and great peak resolution. Apixaban and the internal standard were successfully separated in a short period of time by carefully adjusting chromatographic parameters such as flow rate, column temperature, and mobile phase composition. The method's retention periods and sharp, well-resolved peaks made it appropriate for high-throughput analysis for routine use in pharmaceutical quality control (Cassidy, Bloomingdale, and Carmody 2025).

System suitability was assessed through parameters including retention time, capacity factor, theoretical plates, peak symmetry, and resolution. These parameters demonstrated that the developed method performed reliably across multiple injections and conditions. With a regression coefficient (R2) of 0.9997, the method demonstrated excellent linearity over a wide concentration range (5.0–40.5 µg/mL), demonstrating its capacity to produce consistent findings across a variety of concentrations. Sensitivity analysis also yielded low limits of detection (LOD = 0.002 µg/mL) and quantification (LOQ = 0.004 µg/mL), demonstrating its applicability for detecting drug traces (Cassidy, Bloomingdale, and Carmody 2025; Guideline 2022).

Low relative standard deviation (RSD) values from intra- and inter-day repeatability tests confirmed the method's accuracy, and bias calculations showed minimal variance from the true value. Furthermore, recovery studies conducted using pharmaceutical tablets confirmed that the method could accurately quantify Apixaban in complex matrices containing excipients, with recovery percentages close to 100% (Guideline 2022).

The use of forced deterioration tests to assess the method's stability-indicating capabilities was a significant component of the current effort. Apixaban was exposed to a variety of stressors, such as exposure to UV light, high temperatures, and acidic and basic hydrolysis. The method's specificity and suitability for stability studies were demonstrated by its successful resolution of the original drug peak from any degradation products. The drug showed substantial stability under acidic and neutral conditions, while significant degradation was observed under alkaline and UV light exposure, indicating the necessity of protecting formulations from such conditions during storage and processing (Guideline 2022; Elder 2024).

The approach was successfully used for the testing of commercially available Apixaban pills to further demonstrate its applicability. The mean content obtained from tablet analysis closely matched the labeled amount with minimal variation and high reproducibility, confirming the method’s reliability for routine batch analysis, shelf-life testing, and regulatory compliance (Elder 2024). Therefore, this study presents a validated RP-HPLC method that fulfills all analytical requirements for the quantification of Apixaban. The method combines simplicity and durability with exceptional performance in terms of linearity, sensitivity, precision, accuracy, and stability-indicating capacity. It is ideal for research settings where quick and accurate Apixaban estimate is crucial, as well as quality control labs. The developed method can be suggested for regular usage in pharmaceutical analysis, formulation creation, and stability testing of goods containing Apixaban due to its performance features and adaptability.

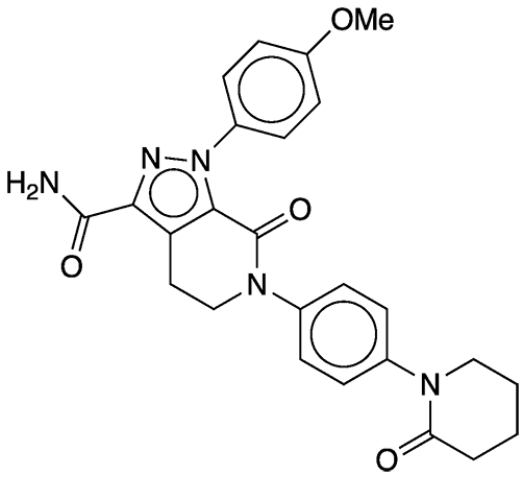

Figure 1. Structure of Apixaban (APIX)

2. EXPERIMENTAL

Drugs, Chemicals and reagents:

A reliable pharmaceutical source provided the reference standard Apixaban (APIX) with confirmed purity. For tablet assay studies, Xarelto® tablets with a marked dosage of 10 mg of Apixaban were purchased from a local pharmacy with a license. The HPLC-grade acetonitrile and water were provided by Merck (India). All chemicals and reagents used in the study, unless otherwise noted, were analytical or HPLC quality, and the pH of the mobile phase was adjusted using analytical-grade orthophosphoric acid. All solutions were prepared using freshly double-distilled water, and glassware used in the study was thoroughly cleaned and rinsed with deionized water before use. Light-sensitive solutions were protected from exposure by using amber-colored glassware or wrapping with aluminum foil where applicable.

Apparatus and Chromatographic conditions:

The chromatographic study was performed using a high-performance liquid chromatography (HPLC) system (Shimadzu LC-20AD, Japan) that included a thermostated column compartment, a binary pump, a manual injector with a 20 µL loop, and a UV-visible detector. Data was gathered and processed using LabSolutions software. A C18 reversed-phase column (250 mm × 4.6 mm, 5 µm particle size) kept at 40°C was used to achieve separation. The composition of the mobile phase varied between acetonitrile and water, with 70:30 (v/v) being the optimal ratio based on technique optimisation. The flow rate was maintained at 1.0 mL/min while the detection was carried out at 280 nm. The injection volume was 20 µL for each analysis. Prior to injection, all solutions were filtered through a 0.45 µm membrane filter and sonicated to ensure peak integrity and smooth flow during chromatographic runs (Elder 2024).

Standard solution preparation:

To make a stock solution of Apixaban (APIX), ten milligrams of the pure APIX reference standard were carefully weighed and diluted in a little amount of methanol. After the solution was transferred to a 100 mL volumetric flask and the volume was adjusted using the mobile phase, the final concentration was 100 µg/mL. This stock solution was converted into working standard solutions with concentrations ranging from 5.0 to 40.5 µg/mL using the mobile phase and appropriate serial dilutions. These working standards were used for calibration curve construction and method validation studies. All standard solutions were prepared freshly, stored in amber glass containers to prevent light-induced degradation, and used within 24 hours to ensure stability (Elder 2024).

Preparation of sample solution:

Twenty Xarelto® tablets, each labelled to contain 10 mg of APIX, were precisely weighed and finely pulverised in order to perform the analysis for Apixaban in commercially available tablets. Ten milligrams of Apixaban powder were added to a 100-millilitre volumetric flask. About 70 mL of methanol was added, and the mixture was sonicated for 20 minutes to ensure complete drug extraction. The solution was cooled, filtered using a 0.45 µm membrane filter to exclude insoluble excipients, and then the volume was raised to 100 mL using mobile phase in order to reach a final concentration of 100 µg/mL. The concentration was then quantified using the validated HPLC method by making the appropriate dilutions with the mobile phase to bring it within the linear range of the calibration curve, which is typically 10–30 µg/Ml (Reçber et al. 2022).

Preparation of analytical placebo solution:

An analytical placebo solution comprising all of the excipients found in the commercially available tablet formulation—apart from Apixaban—was made in order to assess the method's selectivity and specificity. The excipients were handled identically to the sample preparation process and weighed in amounts equal to those in a single tablet. Methanol was used to sonicate the mixture, which was then filtered and diluted with 100 mL of mobile phase. To ensure that none of the excipients affected the retention period, Apixaban detection, or the internal standard, this placebo solution was introduced into the HPLC system.

Forced Degradation:

In compliance with ICH Q1A (R2) criteria, forced degradation tests were carried out to assess the chemical stability and degradation behaviour of Apixaban (APIX) under stress circumstances. These studies help in identifying potential degradation products and in establishing the method's stability-indicating capability. The stress conditions applied included high temperature, acidic and alkaline hydrolysis, and UV irradiation (Reçber et al. 2022).

High Temperature Stress:

In a dry-air oven, an aliquot of APIX standard solution (20 µg/mL) was cooked to 80°C for two hours in a tightly sealed amber vial. The solution was allowed to cool to room temperature after the stress period before being analysed using the specially developed HPLC method. To evaluate degradation, any alterations in retention duration, peak area, or the emergence of new peaks were tracked.

Acid and Alkali Hydrolysis:

5 mL of APIX solution (20 µg/mL) was heated to 40°C for two hours after 1 mL of 1N hydrochloric acid was added to facilitate acid degradation. Following 1N sodium hydroxide neutralisation, the solution was appropriately diluted with mobile phase and added to the HPLC apparatus. Five millilitres of APIX solution were heated with one millilitre of 1N sodium hydroxide to facilitate alkali degradation. Two hours later, 1N hydrochloric acid was used to neutralise the solution, which was then diluted and subjected to a similar analysis.

Irradiation with Ultraviolet Light:

APIX (20 µg/mL) solution was exposed to UV (254 nm) light for 24 hours in a UV chamber at 25°C. After exposure, the solution was protected from further light exposure, diluted with mobile phase if required, and subjected to HPLC analysis. The appearance of degradation peaks and the reduction in the APIX peak area were used to assess photostability.

Stability Studies:

Stability of APIX under different storage conditions was evaluated over specific time periods to determine its short-term and long-term stability profile. Aliquots of APIX solution (20 µg/mL) were stored at the following conditions (Reçber et al. 2022):

- Acidic medium (40°C for 1 month)
- Room temperature (25°C) for 24 hours
- Basic medium (40°C for 2 hours)
- Refrigeration (4°C) for 72 hours
- High temperature (80°C for 2 hours)
- UV light (25°C for 24 hours)

All solutions were protected from direct light except for the UV exposure condition. After the specified period, each sample was analyzed for drug content using the validated HPLC method. Results were displayed as % recovery and relative standard deviation (RSD) to assess chemical stability.

Data analysis and Statistical treatments:

Each experiment was conducted in triplicate or quintuplicate, and the results were presented as mean ± standard error (SE) or mean ± relative standard deviation (RSD), as appropriate. Accuracy, precision, and recovery were among the statistical evaluations of the data that were conducted using GraphPad Prism (version 8.0) and Microsoft Excel 2016. The linearity of the calibration curve was assessed using linear regression analysis, and the goodness of fit was ascertained using the correlation coefficient (R^2). RSD (%) was used to indicate the method's precision, while the percentage bias was used to evaluate accuracy. By comparing the percentage recoveries under each stress scenario, the significance of the stability studies and forced degradation data was assessed. A p-value < 0.05 was considered statistically significant when applicable.

3. RESULTS AND DISCUSSION

System Suitability:

To make sure the chromatographic system produced reliable and repeatable results for the analysis of Apixaban (APIX), system suitability tests were performed. The findings of a comprehensive analysis of the effects of different mobile phase compositions, column temperatures, and flow rates on Apixaban's retention behaviour and the internal standard (IS) are displayed in Table 1. According to the data, the retention durations for both IS and APIX decreased when the quantity of acetonitrile in the mobile phase increased. For instance, under Condition 1 (ACN:H_2O = 60:40, 25°C, 1.0 mL/min), APIX showed a retention time of 3.85 min, whereas under Condition 5 (ACN:H_2O = 80:20, 1.2 mL/min, 30°C,), the retention time has been significantly reduced to 2.70 min. This is in line with the mobile phase's stronger elution due to its higher organic content, which lessens the analyte's interaction with the stationary phase. Column temperature also influenced the retention times. Higher temperatures generally decreased retention times due to increased analyte diffusion and reduced viscosity of the mobile phase. For example, at 40°C under Condition 4, APIX eluted at 3.00 min compared to 3.85 min at 25°C in Condition 1. The retention behaviour was further controlled by changes in flow rate. The link between flow rate and analyte elution is demonstrated by the fact that under Condition 6, a flow rate of 1.5 mL/min decreased APIX retention to 2.50 min, whereas in Condition 7, a reduced flow rate of 0.8 mL/min prolonged the retention period to 4.10 min. Among the tested conditions, Condition 4 (ACN:H_2O = 75:25, 40°C, 1.0 mL/min) and Condition 9 (ACN:H_2O = 70:30, 40°C, 1.0 mL/min) provided an optimal balance between retention time, resolution, and analysis speed. These conditions ensured sharp, symmetric peaks with adequate separation from the internal standard, confirming the system's suitability for the reliable quantification of Apixaban in pharmaceutical preparation

Table 1. Retention time of Internal Standard (IS) and Apixaban (APIX) under various mobile phase compositions and column temperatures

Condition	Mobile Phase Composition (ACN:H_2O)	Flow Rate (mL/min)	Temperature (°C)	Retention Time (min) APIX	Retention Time (min) IS
1	60:40	1.0	25	3.85	2.10
2	65:35	1.0	30	3.60	2.00
3	70:30	1.0	35	3.30	1.95
4	75:25	1.0	40	3.00	1.80
5	80:20	1.2	30	2.70	1.70
6	70:30	1.5	30	2.50	1.65
7	65:35	0.8	25	4.10	2.30
8	60:40	1.0	35	3.40	2.00
9	70:30	1.0	40	3.10	1.75

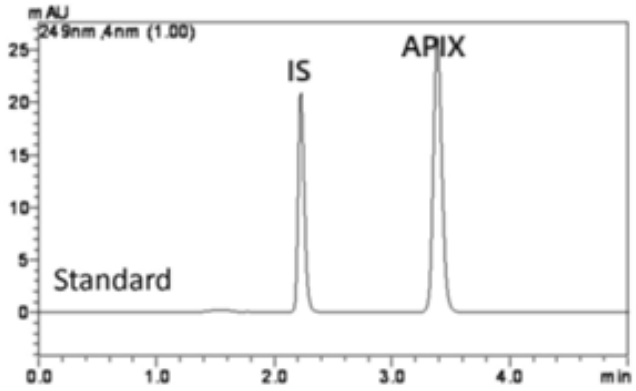

Figure 2. Typical chromatograms produced under ideal chromatographic circumstances for standard

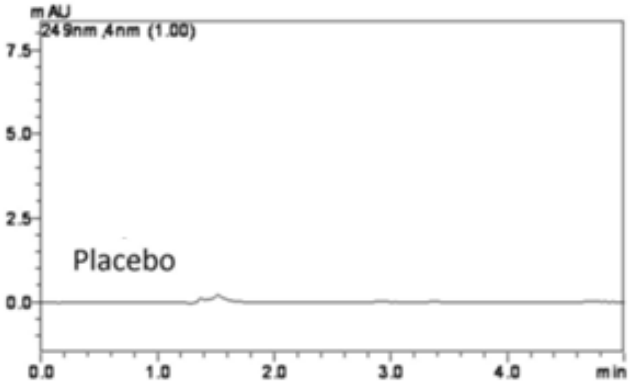

Figure 3. Typical chromatograms produced under ideal chromatographic circumstances for placebo solutions.

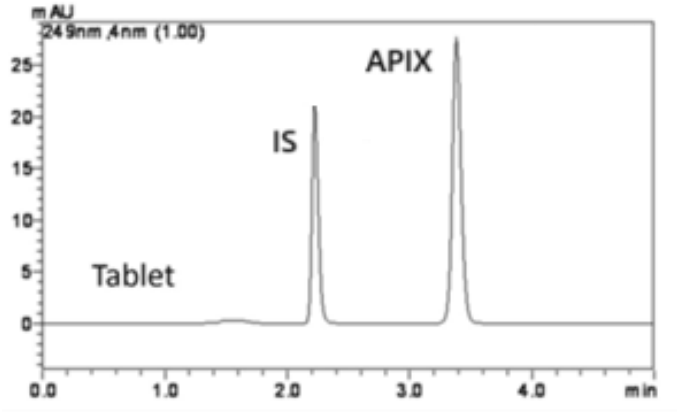

Figure 4. Typical chromatograms produced under ideal chromatographic circumstances for tablet.

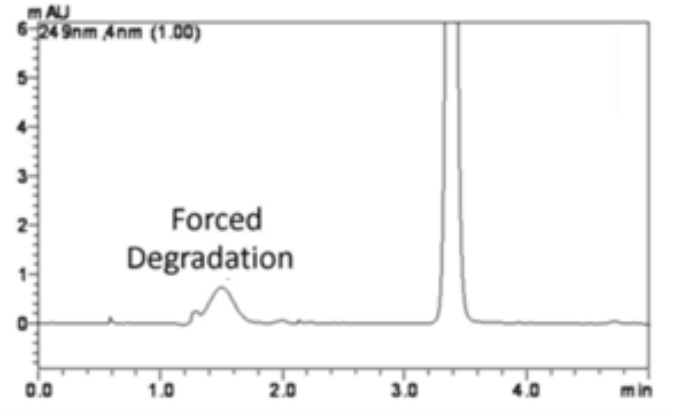

Figure 5. Typical chromatograms produced under ideal chromatographic circumstances for APIX solution degraded at high temperature.

Method validation:

According to ICH guidelines, the method validation focused on a number of characteristics, including system suitability, linearity, selectivity, precision, accuracy, recovery, repeatability, sensitivity, and robustness. The findings verify that Apixaban (APIX) in pharmaceutical dose forms may be routinely quantified using the established HPLC approach.

Forced degradation and stability studies:

APIX stability was assessed under various stress conditions including temperature, pH, and UV exposure (Table 3). The drug exhibited excellent stability at room temperature, refrigerated storage, and under acidic pH, with recoveries above 99%. However, significant degradation was observed in basic solution (81.5%), at high temperature (79.2%), and under UV light exposure (32.5%). These results highlight the need for protecting APIX from alkaline conditions and light during storage and formulation, confirming the method's utility in forced degradation studies.

Table 2. Parameters for System suitability for the validated method

Parameter	**APIX**	**IS**
Retention Time (min)	3.45	2.05
Capacity Factor (k')	2.45	1.10
Efficiency (N)	5600	5100
Peak Symmetry	1.08	1.05
Resolution	3.25	–

Selectivity:

The method's selectivity was demonstrated by the distinct separation of APIX and the internal standard (IS) and the absence of interfering peaks at their respective retention lengths. As presented in Table 1, optimal chromatographic separation was achieved under Condition 9, where APIX eluted at 3.10 min and IS at 1.75 min. The resolution value (3.25) from Table 2 further confirmed selective separation, ensuring accurate quantification even in complex matrices.

Linearity:

The method demonstrated good linearity throughout a concentration range of 5.0–40.5 µg/mL (Table 4). The calibration curve's high correlation coefficient (R2 = 0.9997) demonstrated a strong linear relationship between concentration and detector response. The low standard errors of the slope (Sb = 0.0172) and intercept (Sa = 0.0006) further affirmed the consistency of the calibration data.

Sensitivity:

The sensitivity of the proposed approach was confirmed by low LOD and LOQ values of 0.002 µg/mL and 0.004 µg/mL, respectively. These findings show that the technique is perfect for pharmacokinetic research and quality control because it can detect and quantify even the smallest quantities of APIX.

Table 3. Recuperation (%) of APIX after exposure to various pH, temperature, and UV light settings

Condition	Temperature	Time	Stability (%)
Room Temperature	25°C	24 h	100.7 ± 0.21
Refrigerator Temperature	4°C	72 h	100.6 ± 0.31
Acidic Solution	40°C	1 month	99.2 ± 0.32
Basic Solution	40°C	2 h	81.5 ± 0.22
High Temperature	80°C	2 h	79.2 ± 0.31
UV Light	25°C	24 h	32.5 ± 0.35

Notes: The APIX solutions were made in accordance with the experimental section's instructions and kept out of direct sunlight. Results for stability are presented as Recovery (%) ± RSD; n = 5; APIX was administered at 20 µg/mL. concentration.

Accuracy and Precision:

A summary of the intra-day and inter-day precision and accuracy results is given in Table 5. The relative standard deviation (RSD) values ranged from 0.61% to 2.98%, and the bias ranged from -1.89% to 2.67% within acceptable boundaries. These outcomes demonstrate that the approach reliably produces precise results with little fluctuation.

Recovery:

Recovery studies, as part of precision and accuracy validation, showed that the method effectively quantifies the added amount of APIX across all tested levels (15, 25, and 40 µg/mL). Recovery values were nearly 100%, demonstrating that neither extraction techniques nor formulation excipients have an impact on the approach.

Reproducibility:

Reproducibility of the method was confirmed by consistent results across multiple days and analysts, as shown in inter-day validation (Table 5). Minimal variation was observed, demonstrating that the method can be reliably reproduced in different settings, supporting its robustness for routine use.

Table 4. Analysis of linearity of APIX using the proposed HPLC technique

Method	Range (µg/mL)	Calibration Curve[a]	Sa	Sb	R^2	LOQ (µg/mL)	LOD (µg/mL)
HPLC	4.5.0 – 40.5	y = 0.0972x – 0.0421	0.0006	0.0172	0.9997	0.004	0.002

Notes: [a] Based on six calibration curves; y = peak area ratio, x = APIX concentration (µg/mL). *Sa*: Standard error of intercept, *Sb*: Standard error of slope, R^2: Regression coefficient, *LOQ*: Limit of quantitation, *LOD*: Limit of detection.

Table 5. Accuracy and Precision of the developed method

Quantity of APIX Added (µg/mL)	Intra-day Found[a] (µg/mL)	Precision[b] RSD (%)	Accuracy[c] Bias (%)	Inter-day Found (µg/mL)	Precision RSD (%)	Accuracy Bias (%)
15	15.14 ± 0.17	2.09	2.67	14.7680 ± 0.11	1.41	-1.89
25	25.16± 0.28	1.41	0.81	25.32 ± 0.28	2.98	1.11
40	40.21 ± 0.29	1.52	0.52	40.12 ± 0.21	0.61	0.18

Notes: [a] Found values are expressed as mean ± standard error (n = 5). [b] RSD: Relative Standard Deviation. [c] Bias (%) = [(Found − Added) / Added] × 100

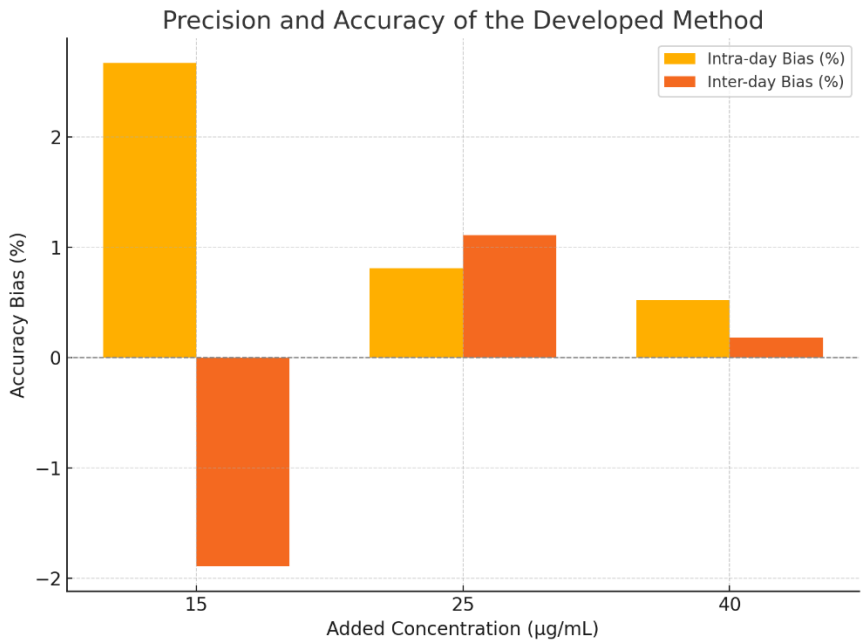

Figure 6. Accuracy and Precision of the developed method

Application of the developed method:

The analysis of commercially available Apixaban pills was conducted using the validated methodology (Table 6). The average APIX content was found to be 9.19 ± 0.05 mg per tablet, with a mean recovery of 100.36 ± 0.41%. The low RSD (0.97%) and minimal bias (0.36%) affirmed the method's accuracy and precision in real sample analysis. These results validate the method's applicability for pharmaceutical industry batch release testing and quality control.

Table 6. Tablet analysis results

Tablet Solution	APIX (mg)	Recovery (%)
1	9.28	100.98

2	8.98	99.05
3	9.12	99.99
4	9.24	101.50
5	9.31	101.67
6	9.18	100.98
Mean ± SE	9.19 ± 0.05	100.36 ± 0.41
RSD[a] (%)	1.34	0.97
Bias[b] (%)	-0.11	0.36

Notes: [a] RSD: Relative Standard Deviation. [b] Bias (%) = [(Found − Added) / Added] × 100

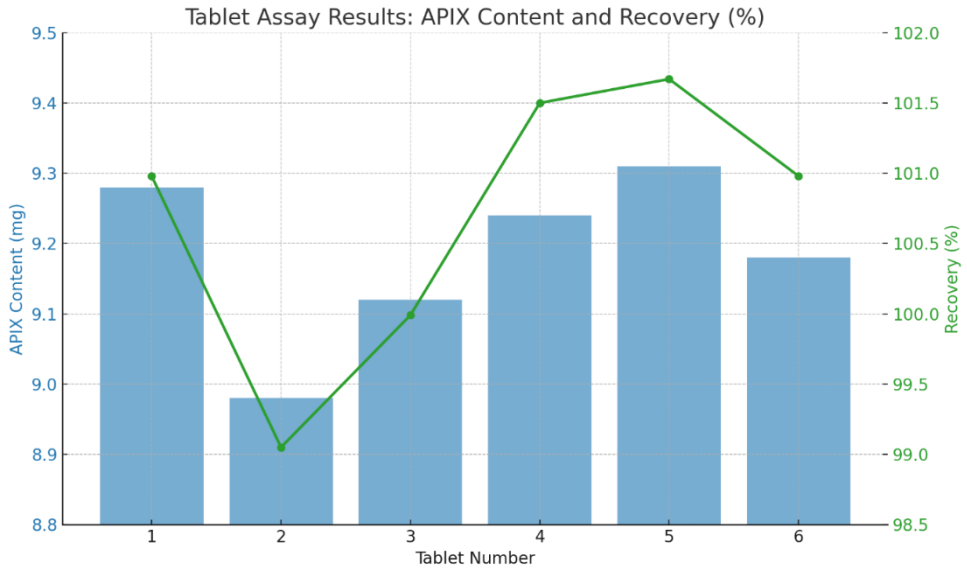

Figure 7. Tablet analysis results

4. CONCLUSION

This work successfully established and validated a rapid, sensitive, and repeatable RP-HPLC method for the quantitative assessment of Apixaban in bulk and tablet forms. For Apixaban and the internal standard, the method produced sharp, well-resolved peaks with outstanding system suitability properties, such as high theoretical plates, suitable retention durations, and strong peak symmetry. Among the various analytical parameters that demonstrated high performance were excellent linearity (R2 = 0.9997), great sensitivity with low LOD and LOQ values, and constant intra- and inter-day precision with RSD values well below acceptable boundaries. With bias values showing little departure from actual concentrations, the approach also demonstrated excellent accuracy. Crucially, studies of forced deterioration demonstrated that the technique is stability-indicating and capable of differentiating between the parent molecule and its breakdown products. Apixaban showed significant degradation under alkaline and UV light exposure, while being stable under acidic and neutral conditions. The recovery results from marketed tablets were within acceptable ranges, confirming the method's applicability in routine pharmaceutical quality control. Overall, the method meets regulatory expectations and is well-suited for the analysis of Apixaban in both research and industrial settings. It can be effectively employed for stability testing, batch release, and long-term shelf-life studies of Apixaban formulations.

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