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Research Article

Quantitative determination of rivaroxaban in pharmaceutical formulations by ultra performance liquid chromatography

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ABSTRACT

Rivaroxaban, which is a factor Xa inhibitor, effectively prevents clot formation in cardiovasculary system. In this study, a novel UPLC method was developed to provide an accurate, sensitive, fast, and reliable way for qualitative and quantitative analysis of rivaroxaban in pharmaceutical dosage forms. Chromatographic separation was achieved using a Phenomenex C18-bonded fused-core silica column (Kinetex® 2.6 µm, 150 mm × 3 mm i.d.). The separation was performed in isocratic mode with a mobile phase consisting of water, acetonitrile, and methanol (55:20:25, *v/v/v*), at a flow rate of 0.5 mL min^{-1}, a column temperature of 40 °C, and a detection wavelength of 249 nm. The method was validated according to ICH Q2(R1) guideline for linearity, range, LOD and LOQ, accuracy, and precision and was successfully implemented to the analysis of rivaroxaban in tablet formulations.

Keywords: Rivaroxaban, Pharmaceutical formulation, UPLC

1. INTRODUCTION

Rivaroxaban (RIV) is an oral anticoagulant used to prevent blood clots, belonging to the oxazolidinone family. It is a potent selective inhibitor of factor Xa, effectively preventing venous thromboembolism in people who have undergone surgery [1,2]. It has high oral bioavailability, predictable pharmacokinetics, a rapid onset of action and strong effect [3]. RIV is clinically effective in the treatment of thromboembolism [4]. RIV is a blood thinner with a molecular formula $C_{19}H_{18}ClN_3O_5S$ and molecular weight 435.882 g mol^{-1} (Figure 1). It has low solubility in organic solvents and is virtually insoluble in water and aqueous media. log P for RIV is 1.74-1.9 with pKa 13.6 (most acidic) and 1.6 (most basic) [5,6].

HPLC is a crucial technique for analysis of pharmaceutical raw materials, by-products, formulations and finished products, in all steps of pharmaceutical production. Advances in the column technology, which is the heart of the technique, provide enhanced chromatographic performance with better limits, analysis performance and peak shapes [7]. In accordance, HPLC seem to remain as the gold-technique in drug analysis, especially for analysis of non-volatile compounds. The ultra performance liquid chromatography method (UPLC), which is evolved from HPLC, is a completely faster

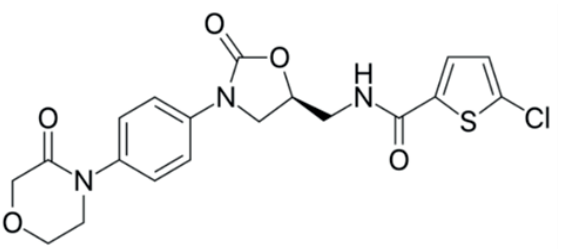

Figure 1. Molecular structure of RIV

system with advanced technology in instrumental components, such as pumps, detectors, autosamplers, etc. This means shorter analysis time, less solvent consumption and higher throughput [8]. UV-Visible Spectrophotometry [9-15], HPLC [2, 16-26], UPLC [27, 28], HPTLC [29, 30] and LC-MS [31, 32] have been published for RIV analysis in pharmaceutical formulations. The fact that UPLC analysis for RIV determination is less common than other ways of analysis has led us to appraise our study into use of different stationary phases and contents. In pharmaceutical field, there is always a need for fast, simple, accurate and sensitive analysis methods. Thus, we developed a simple and rapid UPLC method for the determination of RIV in pharmaceutical tablet formulations. This method addresses the limitations of existing analytical techniques by offering enhanced sensitivity, improved reliability, and greater practicality. Following comprehensive optimization and validation including assessments of accuracy, precision, and linearity the proposed method has proven suitable for quality control of RIV tablets.

2. MATERIALS AND METHODS

2.1. Chemical and Reagents

RIV reference standard with min 99.5% (*w/w*) purity from Molekula (USA) and Escitalopram (ESC, as internal standard) from United States Pharmacopeial Convention (USA) were purchased. Analytical grade chemicals; HPLC gradient grade acetonitrile (ACN), methanol (MeOH) and water for chromatography were obtained from Sigma-Aldrich (Germany).

2.2. Instruments

Analyses were performed using a Shimadzu Nexera-i 2040C 3D series compact ultra performance liquid chromatography system (Japan); chromatograms were processed by using Shimadzu LabSolution Version 5.81 data analysis software.

2.3. Chromatographic Parameters

The mobile phase was composed of water: ACN:MeOH in the ratio of 55:20:25, *v/v/v*. Chromatographic separation was performed using a C18-bonded core shell silica column (Phenomenex Kinetex® 2.6 µm, 150 mm × 3 mm inner diameter). The column oven temperature was set at 40 °C. Samples were injected at 10 µL aliquotes, and flow rate was applied as 0.5 mL/min. To determine the optimal wavelength for analysis, spectra were recorded across a range of 200 to 800 nm. As a result, the maximum absorbance of RIV was determined by UV-Visible Spectroscopy at a wavelength of 249 nm as shown in Figure 2.

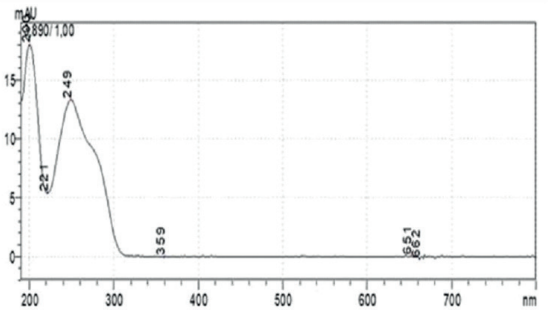

Figure 2. Maximum absorption spectrum of RIV with UV-Visible Spectroscopy

2.4. Preparation of Solutions

MeOH:water (40:60, *v/v*) (dilution mixture) solution was used for all dissolution/dilution procedures. For the preparation of the stock solution I, exactly-weighed 5 mg of RIV was taken and dissolved in dilution mixture in a 10 mL volumetric flask, by mixing with a stirrer for 3 minutes to ensure dissolution. As an intermediate, stock solution II at 100 µg mL^{-1} concentration was prepared by dilution of the stock solution I; stock solution II was used to quantify RIV in both samples and test solutions. ESC was used as internal standart at 50 µg mL^{-1} concentration. An amount of powdered tablets equal to the average tablet weight was also dissolved in 100 mL of the dilution mixture. Resulting mixture was centrifuged at 4000 rpm for 10 min. The supernatant was filtered through a 0.22 µm pore diameter PVDF filter.

2.5. Validation of Analytical Method

2.5.1. Testing of System Suitability

System suitability was recognized as the appropriateness of the instrumentation to analytical quantification requirements. Calculations for

capacity factor (k), tailing factor (T), resolution (Rs), and theoretical number of plates (N) was performed according to United States Pharmacopoeia (USP).

2.5.2. Specificity

In accordance with the ICH Q2(R1) guideline, additional examinations were conducted to evaluate method specificity [33]. Chromatograms and peaks were carefully analyzed to ensure that there was no interference, false peaks, or unexpected signals that could affect the accuracy of the analysis. Peak purity was assessed to ensure that the observed peaks for both the analyte and the internal standard were not the result of co-elution with other compounds. This evaluation confirmed that the detected peaks correspond exclusively to their respective compounds.

2.5.3. Linearity and Range

Seven different levels (9.98, 20, 40, 50, 60, 80 and 99.8 µg/mL) of RIV concentrations were included in a linearity range. Each solution was injected as three times, and the average values were used for further quality control calculations. Linear regression, including both intra-day and inter-day repeats, was used to assess linearity. All statistical calculations were made with GraphPad Prism v6.0b (trial).

2.5.4. Precision

The precision of an analytical method is a measure of the consistency and reproducibility of the results obtained from multiple measurements of the same sample under specified conditions. Reproducibility results are given as standard deviation and relative standard deviation to describe the precision of the method.

2.5.5. Accuracy

Accuracy refers to how the measured values are coles to the accepted reference value. It reflects the extent of any systematic error or bias introduced by the analytical method. In practical terms, accuracy is often assessed through recovery experiments. These involve adding known quantities of the active compounds (analytes) to real sample matrices and measuring how much of the added amount can be recovered. The results are expressed as a percentage of recovery, providing a quantitative measure of the method's accuracy.

2.5.6. Limitations of Detection and Quantification

According to the guidelines established by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH), the LOD and LOQ values were determined based on the standard deviation of the response and the slope of the calibration curve. The detection limit was determined as the concentration corresponding to 3.3 times the signal-to-noise ratio. For the quantification limit, a concentration equivalent to 10 times the noise level was prepared and analyzed in 10 replicates to ensure accuracy and precision.

3. RESULTS AND DISCUSSION

The reported method has been examined and verified with the parameters of system conformity tests, linearity, selectivity, LOD and LOQ, precision and accuracy specified in the guidelines of the International Conference on Harmonization (ICH Q2(R1)). System Suitability Testing (SST) is an important part of analytical method development and validation. It is necessary to determine the most suitable conditions for a good separation of the desired analytes in the analyzes. In determining the most suitable conditions, analyses were conducted to optimize factors such as the mobile phase composition, flow rate, analytical columns, column temperature, detector wavelength, and injection volume. In determining the mobile phase content, it is necessary to provide short elution time and good peak symmetry. In the selection of the mobile phase to be used in the optimization process, analyzes were tried at different rates with water-ACN and water-MeOH mixtures at the beginning. Then, analyses were performed with different ratios of water, MeOH and ACN solvents and the most suitable environment was provided with water:ACN:MeOH (55:20:25, *v/v/v*) mobile phase composition. To determine the mobile phase flow, analyses were performed between 0.3 and 1.0 mL min^{-1}. For the best separation and short analysis time, 0.5 mL min^{-1} was found to appropriate. The result of column temperature variation on the analysis was

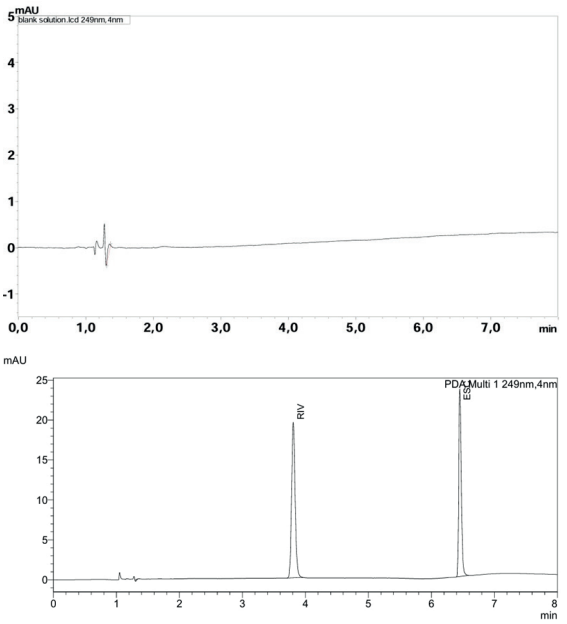

Figure 3. The chromatogram of blank solution and standard solutions (10 µg mL^{-1} for RIV, 50 µg mL^{-1} for ESC)

investigated at temperatures of 25, 30, 40, and 50 °C. It is determined as an operating temperature of 40 °C under optimum conditions. In the preliminary studies carried out with UV-Spectroscopy and UPLC-DAD detector for the determination of the study wavelength, the wavelength that creates a response in accordance with the chemical structure of the substance was determined as 249 nm. With its high absorbance, a wavelength of 200 nm could also be selected for analysis. But it was not chosen because there would be a high fluctuation in the mobile phase line. During the optimization studies, 10 µg mL^{-1} RIV and 50 µg mL^{-1} standard substances were used and the chromatogram is given in Figure 3. The mobile phase flow rate was set to 0.5 mL min^{-1} to achieve low system pressure and optimal elution time.

In addition, the system suitability parameters for the developed method are summarized in Table 1. As a result of the optimised conditions according to USP Pharmacopoeia very suitable results were obtained according to the recommended values.

Linearity was demonstrated at seven different concentrations in the range of 9.98-99.8 µg mL^{-1}. Injection of each solution was carried out in triplicate and linearity was plotted with the mean values obtained. Linearity results were found as slope, intercept, and correlation coefficient as shown in Table 2. The method showed good linearity as 0.9999. LOD and LOQ of the assay were calculated as the ratio of the standard deviation (σ) of the response to the slope (m) of the calibration curve 3.3 and 10. The values of LOD and LOQ for RIV were found to be 1.22 and 3.70, respectively. Recovery studies were carried out with XARELTO®, which contains the active ingredient RIV, which is available in the market. First of all, the RIV standard substance was added to the prepared test solutions with certain quantities. The analyses were carried out at three different concentrations and nine different analyses.

Table 1. System suitability results (n=3)

Parameter	RIV	ESC	Recommended value (USP)
Retention Time (minute)	3.85	6.40	-
Tailing Factor (*T*)	1.2	1.9	0.1<T≤ 1.8
Capacity Factor (*k*)	2.2	4.2	2<k<10
Resolution (R_s)	20.5	27.2	Rs>1.5
Number of theoretical plate (*N*)	19600	12200	N>2000
Repeatability of peak area (*%RSD*)	0.94	0.68	%RSD < 1.0

Table 2. Results of validation studies performed to evaluate method linearity, LOD, LOQ for RIV (n=7)

Parameter	Calculated value
Linearity range (µg mL^{-1})	9.98 - 99.8
Slope±SD (n=7)	5837 ± 117.2
Intercept ±SD (n=7)	1567 ± 24
R^2 (n=7)	0.9999
Limit of detection (S/N=3.3, µg mL^{-1})	1.22
Limit of quantitation (S/N=10, µg mL^{-1})	3.70

Table 3. Accuracy and precision data of RIV

Spiked concentration (µg mL^{-1})	Obtained concentration (µg mL^{-1})± SD; %RSD	Recovery (%)	Bias (%)	Intra-day precision (n = 4) Mean concentration (µg mL^{-1}) ± SD; %RSD	Inter-day precision (n = 4) Mean concentration (µg mL^{-1}) ± SD; %RSD
40	40.41±0.57; 1.41	101.03	1.03	40.05±0.11; 0.28	40.30±0.71; 1.76
50	50.16±0.30; 0.60	100.32	0.32	50.44±0.80; 1.58	49.97±0.28; 0.56
60	59.47±0.67; 1.12	99.12	0.88	60.25±0.78; 1.29	60.47±0.69; 1.15

As a result of the analysis, the results were obtained as given in Table 3.

The developed method demonstrated high precision and accuracy, as shown by intra-day and inter-day analyses of standard RIV solutions at concentrations of 40.0, 50.0, and 60.0 µg mL^{-1}. Precision was confirmed by RSD% values consistently below 2.0, and accuracy was validated with Bias% values also under 2.0. These results highlight the method's reliability and robustness.

Tablet Assay

Tablet samples (XARELTO 10 mg and 20 mg 28 tablet) obtained from BAYER TURK were subjected to analysis to test the applicability of proposed method and to specify rivaroxaban content. Recovery tests were conducted using the conventional addition procedure, with a total of nine independent determinations performed at three different concentrations within the target range. Recovery studies were carried out by adding a certain amount of standard solution mixture while preparing the tablet solutions. In Table 4 and Figure 4 results and values were found to be in accordance with official regulations.

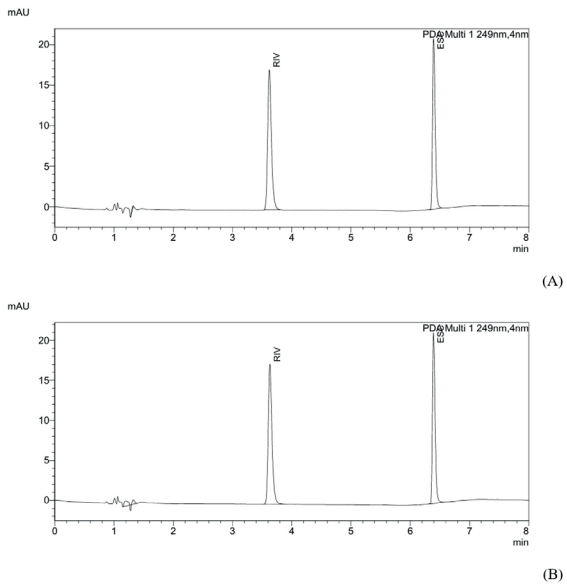

Figure 4. Chromatograms of 10 mg (A) and 20 mg (B) tablet solutions

Table 4. RIV assay in tablets (XARELTO® 10 mg and 20 mg)

Labeled drug claim in tablet (mg)	Determined content of drug (mg)	SD	RSD (%)	Recovery (%)
10	9.98	0.03	0.32	99.80
20	20.02	0.07	0.32	100.10

Results of the determination of RIV in pharmaceutical tablet formulations are shown in Table 4. The accuracies of the UPLC method for 10 mg and 20 mg were 99.80% and 101.10%, respectively, confirming the accuracy of the proposed methods.

4. CONCLUSION

This study developed and validated a rapid, accurate, and precise UPLC method for the qualitative and quantitative analysis of rivaroxaban in pharmaceutical preparations. A simple sample preparation and a short chromatographic study time were used.

Due to the hybrid of the liquid chromatography device used in the developed method, it is advantageous both in terms of working at high pressures and using larger diameter columns compared to other UPLC devices. In this way, analysis is carried out in a short time and allows less consumption of the chemicals used.

The linearity test results show that there is a good correlation between $R \geq 0.9999$ and the peak area and concentration for rivaroxaban and that the calibration curve is method linear over the studied concentration range. In addition, reproducibility and accuracy criteria are provided. The low retention time and the values of the detection limit (1.22 µg mL^{-1}) and quantification limits (3.70 µg mL^{-1}) show the superiority of the method over other methods.

The proposed liquid chromatography method stands out with its speed, simplicity, use of environmentally friendly solvents and low solvent consumption compared to similar studies in the literature. These features make the method more reliable and sustainable. For these reasons, the proposed method will greatly simplify the work of analysts in quality control laboratories, particularly for the analysis of formulations and finished products. It will also provide information for advanced analysis methods related to RIV.

Ethical approval

Not applicable, because this article does not contain any studies with human or animal subjects.

Author contribution

Conceptualization, M.K. and N.Ö.C.; Methodology, M.K. and N.Ö.C.; Software, M.K.; Validation, M.K. and N.Ö.C.; Formal analysis, M.K. and N.Ö.C.; Investigation, M.K.; Resources, M.K.; Data curation, N.Ö.C.; Writing—original draft preparation, M.K. and N.Ö.C.; Writing—review and editing, M.K.; Visualization, M.K. and N.Ö.C.; Supervision, N.Ö.C.; Project administration, N.Ö.C.; Funding acquisition, N.Ö.C. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declared that there is no conflict of interest.

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