

Development and Validation for Quantitative Estimation of rivaroxaban by RP-HPLC with Cardiovascular Effects**Bhawna Lakhawat¹, Akash², Avinash Bairwa³**¹PhD Scholar, Department of Pharmacology, Index Medical College Hospital and Research Center, Indore, M.P., India²Associate Professor, Department of Pharmacology, Index Medical College Hospital and Research Center, Indore, M.P., India³PhD Scholar, Department of Biochemistry, Index Medical College Hospital and Research Center, Indore, M.P., India

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Abstract

Background: A simple RP-HPLC method has been developed and validated quantitative estimation of Rivaroxaban in formulation. It is a highly selective direct Factor Xa inhibitor with excellent oral bioavailability and rapid onset of action. The detection was carried in the room temperature at 249 nm. From the trials, potassium dihydrogen orthophosphate buffer: ACN was selected as a mobile phase for the proposed method. The 60:40 v/v ratios of selected mobile phases resulted in asymmetric peak. The buffer at pH 3 with buffer strength of 0.1M was ideal for the determination of rivaroxaban. The method was linear in the concentration range of 1-5 µg/ml with correlation coefficient (r²) of 0.9996. The mean recovery results of assay were good and the label claim of rivaroxaban tablets was found to be satisfactory.

Keywords: RP-HPLC and Rivaroxaban.**DOI:** 10.25258/ijcpr.18.4.303

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Introduction

Rivaroxaban, dabigatran etexilate, and apixaban are all approved in Europe and the United States for the prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation (AF) who are at risk of stroke [1-3]. Rivaroxaban is approved for the prevention of atherothrombotic events in patients with recent acute coronary syndrome (ACS) and elevated cardiac biomarkers, in combination with standard antiplatelet therapy [4].

Rivaroxaban is orally active direct factor Xa inhibitor approved by CDSCO for the prophylaxis treatment of venous thromboembolism. Rivaroxaban is chemically 5-Chloro-N-((5S)-2-oxo-3-[4-(3-oxo-4-morpholinyl) phenyl]-1,3-oxazolidin-5-yl) methyl)-2-thiophene- carboxamide [1]. Rivaroxaban is available in India in the brand. It is a highly selective direct Factor Xa inhibitor with excellent oral bioavailability and rapid onset of action [5]. There are no official methods for the assay of Rivaroxaban in any pharmacopoeia. The literature review revealed few methods of estimation of Rivaroxaban in its formulation by UV spectroscopy [6-7] and HPLC [8-9].

Materials and Methods

HPLC grade solvents like methanol, dimethyl sulphoxide and acetonitrile will supply Chemicals. Rest of the chemicals and reagents used will supply by Ranbaxy chemicals. Dialysis membrane tubes and bovine serum albumin (BSA) obtained from Hi-Media lab. Formulations containing the selected cardiovascular drugs were procured from pharmacy. In Dimethylsulphoxide (DMSO) the drug was found to be completely soluble thus it was selected to prepare the stock solution. Appropriate concentration was prepared by dilution with ACN and the solution was scanned. The normal spectrum of rivaroxaban was recorded and the absorption maximum was observed at a wavelength of 249 nm.

Preparation of stock and working standard solutions: The stock solution was prepared by transferring accurately weighed 10mg of rivaroxaban in 10ml volumetric flask. It was dissolved with little amount of DMSO and the volume was made up to mark. The serial dilutions were done with ACN to get preferred concentration of rivaroxaban for further analysis.

Method validation: The method validation studies were carried out and the parameters are described below;

Linearity: The various concentrations of working standard solutions were prepared and it was scanned against blank. The $\lambda_{\max}$ of 249 nm was selected to record absorbance of the solutions. Calibration parameters were analyzed by plotting calibration graph and the optical characteristics were also calculated.

Specificity: The spectra obtained from the sample solution in presence of excipients were compared with that of standard solutions for any change in the spectra due to the presence of excipients.

Accuracy: It was evaluated by standard addition method. Standard were added and the absorbance ratio was calculated by comparing the pre-analyzed sample.

Precision: It was determined by intra-day and inter-day precision studies. Three different concentrations of drug solution in the linearity range were prepared and absorbance was recorded. For intra-day studies, measurements were repeated

Detection limit and quantitation limit (LOD and LOQ) The Detection Limit and Quantitation Limit can be calculated based on the Standard deviation of the response and the Slope. The results obtained.

Stability of solution: The solution stability was analyzed by recording the absorbance of standard solution stored under normal and refrigerated conditions ($8^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$). The percentage decrease in absorbance of the solutions was measured in different time intervals.

Results

The selection of mobile phase was done by carrying out chromatographic analysis with different mobile phase compositions. From the trials, potassium dihydrogen orthophosphate buffer: ACN was selected as a mobile phase for the proposed method. The 60:40 v/v ratios of selected mobile phases resulted in asymmetric peak. The buffer at pH 3 with buffer strength of 0.1M was ideal for the determination of rivaroxaban.

Linearity: Results obtained from linearity study reveals linear concentration range of 1 to $5\mu\text{g/ml}$.

Under the experimental conditions described above, linear calibration curve obtained throughout the concentration ranges study. Table-1 The regression equation reveals slope of 32567.6 and intercept of 7478.8. The correlation coefficient (r^2) was found to be 0.9996. Figure-1

Accuracy: The accuracy of the method was determined by estimating recoveries of rivaroxaban. The calculated mean percentage recoveries of rivaroxaban are tabulated. The mean percentage recovery value of >98 reveals the method is accurate for the estimation of rivaroxaban.

Precision: Repeatability, intra-day and inter-day precision studies were performed. The results of precision studies of rivaroxaban are given in table The %RSD value of <2 describe the method precision Table-1.

LOD and LOQ: the detection limit and quantitation limit calculated based on the standard deviation of the response and the slope. The calculated LOD of rivaroxaban was found to be $0.03223\mu\text{g/ml}$ and LOQ was found to be $0.1015\mu\text{g/ml}$ Table-3.

Table 1: Linearity data for rivaroxaban

S. No	Concentration ($\mu\text{g/ml}$)	Peak area
1	1	37929
2	2	69846
3	3	102134
4	4	152176
5	5	178634

Table 2: Inter-day & Intra-day Precision of Rivaroxaban

Conc. ($\mu\text{g/ml}$)	Intra-day		Inter-day	
	Mean Conc. $\pm$ SD*	% RSD*	Mean Conc. $\pm$ SD*	% RSD*
3	3.0926 ± 0.0169	0.4997	2.9949 ± 0.0106	0.3567
4	3.0143 ± 0.0506	1.2489	4.0956 ± 0.0251	0.6432
5	5.0125 ± 0.0239	0.4877	5.0171 ± 0.0297	0.7892

Table 3: LOD and LOQ of Rivaroxaban

Limit of Detection concentration in $\mu\text{g/ml}$	Limit of Quantitation concentration in $\mu\text{g/ml}$
0.03223	0.1015

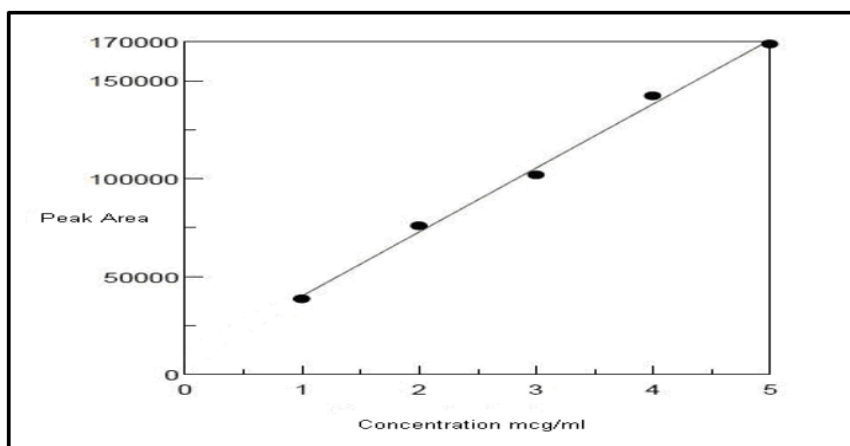


Figure 1: Linearity graph of rivaroxaban

Discussion

The plot of peak areas against concentrations was evaluated by linear regression method and good linearity of rivaroxaban was observed in concentration range of 1 to 5 µg/ml with correlation coefficient of 0.9996. The method precision was proved by the better results of intra-day and inter-day precision. Adequacy of the proposed method for determination of rivaroxaban was assured by ideal system suitability parameters, robustness and specificity.

The recoveries and stability of the rivaroxaban were found to be excellent; thus, the validated HPLC method was suitable for the analysis of formulation. The mean recovery results of assay were good and the label claim of rivaroxaban tablets was found to be satisfactory. Binding of rivaroxaban in presence of clopidogrel was found to be 90.5%. The displacement of rivaroxaban from protein was found to be 6.5%. The results demonstrate considerable amount of displacement of rivaroxaban from protein by clopidogrel.

The present study developed and validated a simple and reliable RP-HPLC method for the quantitative estimation of rivaroxaban in tablet formulations. The optimized method employed potassium dihydrogen phosphate buffer and acetonitrile in a 60:40 (v/v) ratio, with a buffer pH of 3.0 and detection at 249 nm. The method demonstrated linearity over the concentration range of 1–5 µg/mL with an excellent correlation coefficient ($r^2=0.9996$). Mean recovery was greater than 98%, while intra-day and inter-day precision showed % RSD values below 2%, indicating satisfactory accuracy and precision.

The findings of the present study are consistent with recent investigations demonstrating the suitability of RP-HPLC for rivaroxaban analysis. Jahangir et al. developed a validated RP-HPLC-PDA method for determination of rivaroxaban in pharmaceutical formulations and human plasma. Their method showed excellent linearity with an r^2 of 0.9996 and

was reported to be precise, accurate, robust and rugged. The authors also demonstrated specificity under photolytic, thermal, oxidative, acidic and basic stress conditions¹⁰. The comparable correlation coefficient obtained in the present study ($r^2=0.9996$) further supports the suitability of RP-HPLC for quantitative rivaroxaban determination.

A stability-indicating RP-HPLC method reported by Rao et al. provided more extensive separation of rivaroxaban and its related substances. Their method achieved baseline separation of rivaroxaban from fifteen impurities and was validated for precision, accuracy, linearity, LOD, LOQ and robustness [11]. This demonstrates the increasing importance of chromatographic methods not only for assay determination but also for monitoring pharmaceutical impurities and degradation products.

Similarly, a study published in 2022 developed a stability-indicating HPLC method capable of separating rivaroxaban from its process-related impurities and degradation products.

The method demonstrated satisfactory specificity, precision, accuracy, linearity and robustness and was proposed for quality-control analysis of commercial products [12]. Compared with these more comprehensive impurity-oriented methods, the present investigation primarily focuses on quantitative estimation of rivaroxaban in tablet formulations, with emphasis on routine analytical validation.

The linearity observed in the present study over 1–5 µg/mL was associated with a strong concentration-response relationship. The peak area increased progressively from 37,929 at 1 µg/mL to 178,634 at 5 µg/mL, and the resulting calibration curve demonstrated excellent linearity. Recent work by Mestarechi et al. [13] also demonstrated excellent linearity for rivaroxaban using RP-HPLC, with an r^2 of 0.999 over a considerably broader concentration range of 50–1000 ppm. Although the concentration ranges differed substantially, both studies

demonstrate a strong linear relationship between rivaroxaban concentration and chromatographic response. The precision results obtained in the present investigation were also satisfactory. Intra-day %RSD ranged from 0.4877–1.2489%, whereas inter-day %RSD ranged from 0.3567–0.7892%, remaining below the commonly accepted 2% criterion.

These findings are comparable with recent stability-indicating methods in which precision and accuracy were also reported within acceptable analytical limits. Mestareehi et al. reported %RSD values below 2% for precision and accuracy, while recovery values were within an acceptable range, confirming good reproducibility of their RP-HPLC method [13,14].

The sensitivity of the present method was demonstrated by an LOD of 0.03223 µg/mL and an LOQ of 0.1015 µg/mL. Recent stability-indicating methods have demonstrated even greater sensitivity for impurity and degradation-product analysis. Mestareehi et al. reported an LOD of 0.3 ppm and LOQ of 1 ppm for impurities and degradants, whereas their method was specifically designed to identify low-level degradation products [14].

These differences are expected because the analytical objectives and concentration ranges of impurity profiling methods differ from those of routine assay methods. More recently, Nimje et al. developed a stability-indicating RP-HPLC method for rivaroxaban tablets using a C18 column, water:acetonitrile (45:55, v/v) mobile phase and detection at 249 nm.

Their method demonstrated satisfactory validation characteristics and was capable of detecting degradation products generated under acidic, alkaline and oxidative stress conditions [15]. The use of the same detection wavelength (249 nm) in that study and the present investigation provides additional support for the suitability of this wavelength for rivaroxaban estimation.

The present study also investigated the interaction of rivaroxaban with clopidogrel. Protein binding of rivaroxaban in the presence of clopidogrel was reported as 90.5%, with 6.5% displacement of rivaroxaban from protein.

This finding indicates displacement under the experimental conditions; however, an in-vitro protein-displacement observation should not be interpreted alone as evidence of a clinically significant drug-drug interaction. Overall, the present study demonstrates that the proposed RP-HPLC method possesses satisfactory linearity, accuracy, precision, sensitivity, specificity and robustness. Recent literature further supports the application of RP-HPLC for rivaroxaban assay, stability assessment and impurity profiling. The

proposed method may therefore be useful for routine quality-control analysis of rivaroxaban tablet formulations.

Conclusion

In this present study, fast and reliable RP-HPLC method was developed and validated for the determination of Rivaroxaban in tablet formulation. The proposed method has the lowest limit of detection values and is more sensitive method. From the results obtained, we concluded that the suggested methods showed high sensitivity, accuracy, and specificity. This method is simple and inexpensive and can be employed for the routine quality control of Rivaroxaban tablet formulation in cardiovascular effects.

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