

Research Article



Novel Validated RP-HPLC Method for Determination of Rivaroxaban in Bulk and its Pharmaceutical Dosage Form

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Received: 11-06-2020; Revised: 16-08-2020; Accepted: 23-08-2020.

DOI: 10.47583/ijpsrr.2020.v64i01.033

ABSTRACT

A simple, specific, accurate, and precise reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the quantification of Rivaroxaban in Bulk drug and Pharmaceutical dosage form. The quantification is carried out with a Nucleosil C18, (250 × 4.6 mm, 5 μm) column maintained at room temperature with mobile phase consisted of a mixture of acetonitrile and water in the ratio of 50:50 (v/v) delivered at a flow rate of 1.0 ml / min and effluents were monitored at 251 nm through PDA detector. The total run time was set at 7 min. The retention time of Rivaroxaban was found to be 4.893 min. The method was validated as per ICH guidelines, parameters like linearity, accuracy, precision, specificity, limit of detection, limit of quantification and robustness. The linearity was in the range of 5-50 μg/ml with correlation co-efficient of 0.9992. The recovery of rivaroxaban was found to be 98.84 %. Limit of detection and limit of quantification were found 0.054 μg/ml and 0.164 μg/ml respectively. The proposed method was successfully applied for the quantitative determination of Rivaroxaban in tablet dosage form in quality control testing laboratories.

Keywords: Rivaroxaban, HPLC, Method validation, Tablets dosage form.

INTRODUCTION

Rivaroxaban (RIV; Figure 1) is an oral oxazolidinone-based anticoagulant; it is a potent and selective direct inhibitor of factor Xa for the prevention of venous thromboembolism in adult patients after total hip replacement or total knee replacement surgery.¹ Chemically it is 5-chloro-N-[[[(5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl) phenyl]-1, 3-oxazolidin-5-yl] methyl] thiophene- 2-carboxamide. It is an empirical formula C₁₉H₁₈Cl N₃O₅S and molecular weight of 435.882 g/mol.²⁻³

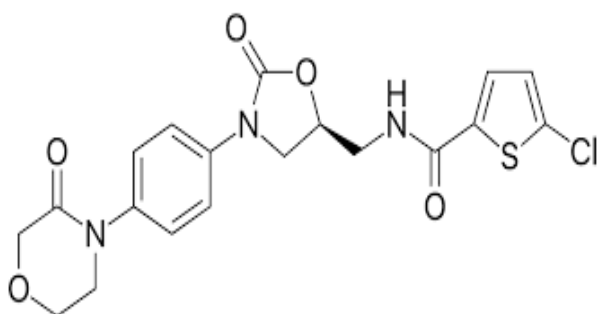


Figure 1: Chemical structure of Rivaroxaban

Literature survey revealed that few analytical methods have been reported for estimation of Rivaroxaban individually or in combination with other drugs. The reported methods are Spectrophotometric⁴, RP-HPLC⁵⁻¹⁷ and HPTLC¹⁸ methods. The present study was aimed to develop a simple, sensitive, rapid and precise RP-HPLC method for estimation of Rivaroxaban. The analytical method was validated according to ICH validation parameters.¹⁹⁻²¹

MATERIALS AND METHODS

Chemicals and reagents

Pure sample of Rivaroxaban obtained from Hetero drugs private ltd, Hyderabad, India. The marketed formulation of Xarelto-10 mg tablets (Rivaroxaban 10 mg/tablet) Manufactured by Bayer Zydus AG, Germany, imported and marketed by Bayer Zydus Pharma Limited, Maharashtra, India were procured from local pharmacy store. HPLC grade of acetonitrile were procured from SD Fine Chemicals Ltd., Mumbai, India. Methanol of HPLC grade was purchased from Merck, Mumbai, India. HPLC grade water was obtained by double distillation and purified additionally with Milli-Q water purification system.

Instrumentation

The analysis was performed by using a chromatographic system, Agilent HPLC comprised of a 1260 Quaternary pump, a 20 μL injection loop and a 1260 photo diode array detector and running on E-Z Chrome software with a reverse phase Nucleosil C₁₈ column having 250 x 4.6 mm internal diameter, 5 μm particle size. UV-Visible Spectrophotometer (Elico SL-210-) Shimadzu electronic balance (AX-200) was used for weighing purpose.

Chromatographic Conditions

Rivaroxaban was analyzed with Nucleosil C₁₈ column (250 x 4.6 mm, 5 μm particle size) for the chromatographic separation and column was maintained at ambient temperature. The mobile phase was composed of a mixture of acetonitrile and water in the ratio of 50:50 v/v and it was delivered at a flow rate of 1.0 mL/min and detection was monitored at 251 nm with PDA detector.

Mobile phase was used as diluent. Injection volume was 20 μ l. The run time was 7 min. The retention time of Rivaroxaban was found to be 4.893 min.

Table 1: Optimized chromatographic conditions

Parameter	Condition
Mobile phase and ratio	Acetonitrile: Water (50:50 % v/v)
Column	Nucleosil C ₁₈ 250 mm×4.6 mm; 5.0 μ m
Wave length	251nm
Column Temperature	Ambient
Injection volume	20 μ L
Flow rate	1.0 min/ml
Run Time	7 minutes

Preparation of Standard stock solution

Accurately weighed and transferred 10 mg of Rivaroxaban working standard into 10 ml clean and dry volumetric flask, add 5 ml of Acetonitrile and sonicated for 5 minutes and made up to the final volume with diluent. From the above stock solutions, 1 ml was pipette out in to a 10 ml volumetric flask and then made up to the final volume with same diluent, and it gives 100 μ g/ml solution.

Preparation of Sample Solution

Accurately 20 tablets were weighed individually and the average weight was calculated and powdered. The tablet powder equivalent to 10 mg of Rivaroxaban and it was transferred into a 10 ml volumetric flask, to that 5 ml of diluent was added and sonicated for 5 minutes at controlled temperature to dissolve the powder, further the volume was made up with diluent, and filtered through 0.45 μ membrane filter. From this solution 1 ml was diluted to 10 ml with the same diluent to give a concentration of 100 μ g/ml of Rivaroxaban.

Method Development and Optimization

The optimized HPLC conditions several mobile phases of different compositions were tested to develop an optimization of chromatographic conditions such as tailing factor, good peak shape, and theoretical plates. For the selection of the mobile phase primarily methanol: acetonitrile, methanol: water, acetonitrile: water has been tested for different compositions, flow rates and ratios. Finally, mobile phase consisting a mixture of acetonitrile: water (50:50 % v/v) at a flow rate of 1.0 ml/min was found to be satisfactory and proper system suitability parameter results were obtained.²²

Method Validation

The method was validated for Specificity, linearity, accuracy, precision, limit of detection, limit of quantification and robustness by following procedures.

System Suitability

System suitability is an integral part of the chromatographic system. It is verification of resolution, capacity factor, tailing factor, theoretical plate count, relative retentions etc. are calculated and compared with standard specification of system.²²

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. The specificity of an analytical method is to determine the effect of excipients and other additives that are generally present in the formulation. The test results obtained were contrasted with the results of standard drug.²³

Linearity

Linearity is the ability (within specified range) to obtain test results are directly proportional to the concentration of analyte in the sample. Linearity is evaluated by visual inspection of plot of signal as a function of analyte concentration. If there is a linear relationship test results are calculated by regression line by method of least squares.²³

The linearity of the method was determined at six concentration levels ranging from 5-50 μ g/ml for Rivaroxaban. Evaluation of the drug was performed with PDA detector at 251 nm, peak area was recorded for all the peaks. The correlation coefficient value of Rivaroxaban was 0.9992. The results shown that an excellent correlation exists between peak area and concentration of drug within the concentration range indicated.²³

Range

The range of analytical procedure is the interval between the upper and lower concentration of analyte in the sample.²³

Accuracy

Accuracy of analytical method is 'measure of how close the experimental value to the true value' accuracy of the method was determined by standard addition method. A known amount of standard drug is added to the fixed amount of pre-analyzed injection solution. Percent recovery is calculated by comparing the area before and after addition of the standard drug. The standard addition method is performed at 10 %, 30 % and 50 % level. The solutions are analyzed in triplicate at each level as per the proposed method.²³

The accuracy of the method was determined by calculating recovery of Rivaroxaban by the method of standard addition. Known amount of Rivaroxaban was added to a pre-quantified sample solution and the amount of Rivaroxaban was estimated by measuring the peak area ratios and by fitting these values to the straight-line equation of calibration curve. The recovery studies were carried out three times over the specified concentration

range of 10%, 30% and 50% levels. The amount of Rivaroxaban was estimated by measuring the peak area ratios by fitting these values to the straight-line equation of calibration curve. From the above determination, percentage recovery and standard deviation of percentage recovery were calculated.²³

Precision

The closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. The intra-day precision study of Rivaroxaban was carried out by estimating the correspondence responses six times on the same day with 100 % concentration and inter-day precision study of Rivaroxaban was carried out by estimating the correspondence responses six times next day with 100 % concentration.²³

Limit of detection and Limit of Quantification

Limit of detection (LOD) is defined as the lowest concentration of analyte that gives a detectable response. Limit of Quantification (LOQ) is defined as the lowest concentration of analyte that can be quantified with a specified level of accuracy and precision. For this study, six replicates of the analyte at lowest concentration are measured and quantified.

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solution using the developed HPLC method.²³

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters. The robustness of the proposed method is estimated by changing flow rate of the mobile phase and composition of the mobile phase.¹⁹

RESULTS AND DISCUSSION

The HPLC procedure was optimized with a view to develop an accurate assay method for the determination of Rivaroxaban in bulk and pharmaceutical dosage form by using Nucleosil C18 (250 x 4.6 mm internal diameter; 5 µm particle size) column with mobile phase of acetonitrile and water in the ratio of 50:50 v/v. The flow rate of mobile phase at 1.0 ml/min and the component was monitored and detected with PDA detector at 251 nm. The eluted drug peak with good shape and well resolved. The results of optimized chromatographic conditions were shown in Table 1. The retention time, number of theoretical plates

and tailing factor of Rivaroxaban was found to be 4.893 min., 13924 and 1.002 respectively, which indicates efficient performance of the column. The method was linear in the range of 5-50 µg/ml for Rivaroxaban with correlation coefficient of 0.9992. The regression equation of Rivaroxaban concentration over its peak area ratio was found to be $Y=147199x$, where X is the concentration of Rivaroxaban and Y is the respective peak area. The linearity results were shown in Table 2 and Figure 2.

Table 2: Linearity results of Rivaroxaban

S. No	Concentration (µg/ml)	Retention time (min)	Peak area (mv.s)
1	5	4.877	666301
2	10	4.893	1447271
3	20	4.790	2797055
4	30	4.800	4199852
5	40	4.890	6006383
6	50	4.887	7465521

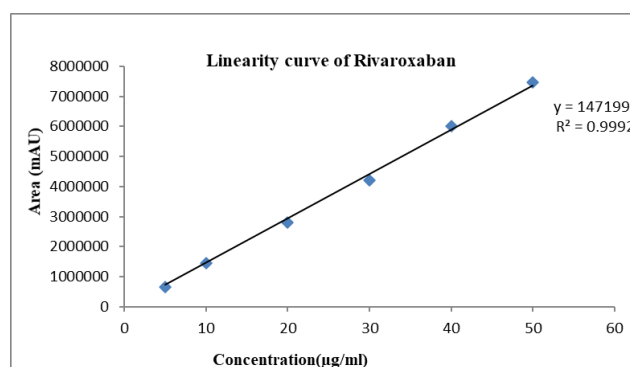


Figure 2: Linearity curve of Rivaroxaban

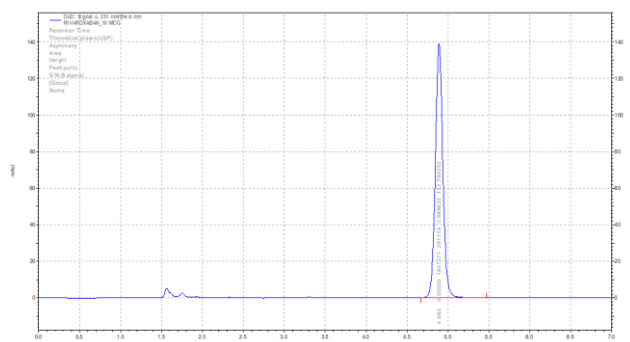


Figure 3: Chromatogram of Rivaroxaban

The mean % recoveries was found to be 98.84 % which indicate the method is accurate. The accuracy results were shown in Table 3. The % RSD for intra-day precision and inter-day precision for Rivaroxaban were found to be 0.18 and 0.20, the values were less than 2 % which indicate the method is precise. The results of precision studies were shown in Table 4. The limit of detection (LOD) and limit of quantification (LOQ) for Rivaroxaban were found to be 0.054 µg/ml and 0.164 µg/ml, which indicate the sensitivity of the method.

Table 3: Accuracy result of Rivaroxaban

Spike Level	Amount added (µg/ml)	Amount found (µg/ml)	% recovery	mean % recovery
10%	10	9.91	99.1	98.20
10%	10	9.78	97.8	
10%	10	9.75	97.5	
30%	30	9.97	99.7	99.64
30%	30	9.94	99.4	
30%	30	9.97	99.7	
50%	50	9.78	97.8	98.6
50%	50	9.92	99.2	
50%	50	9.89	98.9	

The robustness was performed for the flow rate variations from 1ml/min to 1.2 ml/min. The method is robust only in less flow condition $\pm 2\%$. The results are summarized on evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ± 0.2 ml/min. The method is robust only in less flow condition. The typical variations studied under this parameter was mobile phase flow rate. Overall % RSD was found to be less than 2% for all the variations which indicates that the proposed method is robust.

Table 4: Intraday and Inter day Precision Results of Rivaroxaban

S. No.	Intraday Precision		Inter day Precision	
	RT	Peak Area	RT	Peak Area
1	4.800	4199852	4.800	4199763
2	4.801	4198763	4.801	4198789
3	4.801	4192314	4.801	4192216
4	4.801	4189216	4.801	4188216
5	4.800	4186413	4.800	4186512
6	4.800	4179897	4.800	4176342
Average		4191076		4190306
Std. Dev		7587.0		8704.0
% RSD		0.18		0.20

The summary of system suitability parameters and validation parameters were shown in Table 5. Validated method was applied for the determination of Rivaroxaban in commercial tablet formulation that was obtained by injected 3 replicates of the sample solutions. The amount of drug and percentage of assay was found to be 14.84 mg/tablet and 98.933%. Typical chromatogram of drug Rivaroxaban was shown in Figure 3. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in the formulation did not interfere with the estimation of the drug by the proposed method.

Table 5: System Suitability and Validation Parameters

S. No.	Parameter	Results
1	Linearity range (µg/ml)	5-50
2	Correlation coefficient (r^2)	0.9992
3	Retention times (min)	4.893
4	Theoretical plates (N)	13924
5	Tailing factor	1.002
6	Mean % recovery (%)	98.84
6	Repeatability (% RSD)	0.18
7	Reproducibility (% RSD)	0.20
8	LOD (µg/ml)	0.054
9	LOQ (µg/ml)	0.164
10	Robustness (% RSD) (1ml/min & 1.2 ml/min)	0.239 0.180
11	Assay (%)	98.993

CONCLUSION

Proposed Study describes new HPLC method for the estimation of Rivaroxaban bulk and in its tablet formulation. The method was validated and found to be simple, sensitive, accurate, precise and robust. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the proposed method can be used for routine analysis of estimation of Rivaroxaban in regular quality control testing laboratories.

Acknowledgements: The authors are thankful to Management of Vignan Pharmacy College, Vadlamudi, India for providing necessary facilities for carryout the research work. And authors also thankful to Hetero drugs private ltd, Hyderabad, India for providing the working standard drug Rivaroxaban.

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Source of Support: None declared.

Conflict of Interest: None declared.

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