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Research Article

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RP-HPLC Method Development and Validation for Determination of Rivaroxaban in the Pure and Pharmacuetical Dosage Form

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ABSTRACT

A simple, rapid, accurate, precise, robust and reproducible reverse phase high performance liquid chromatographic method was developed for the determination of Rivaroxaban in pure drug and pharmaceutical dosage form. The quantification was carried out using enable C_{18}(250×4.6mm,5µm) column in a binary mode with mobile phase comprising 0.1%glacial acetic acid: acetonitrile in 30:70 %v/v at flow rate 1ml/min, detection was carried out at 250nm using PDA detector with injection volume 20µl, the retention time was found to be 3.44min. The proposed method was validated as per ICH Q2B guidelines. The method produced linear response in the concentration range of 2-10µg/ml (R^2 ˜0.9993). The recovery studies were carried out and found to be within 98-102%. %RSD was found to be 2%. LOD and LOQ of Rivaroxaban for the method were found to be 0.008µg/ml and 0.248µg/ml respectively. The proposed method was statistically evaluated and can be applied for the routine analysis, quality control of raw materials, formulation of different strengths, dissolution studies and bioequivalence studies for the same formulation of Rivaroxaban.

Keywords: Rivaroxaban; Acetonitrile; RP-HPLC; Validation; ICH guidelines

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INTRODUCTION

Rivaraxoban is 5-chloro n-{[(5S)-2-oxo-3-[4-(3-xomorpholin-4-yl) phenyl]-1, 3-oxozolidin-5-yl] methyl} thiophene-2-corboxamide, [1]. It belongs to the class of direct factor Xa inhibitor approved for the prevention of venous thromboembolic events in patients who have undergone total hip or total knee replacement surgery. RXN blocks the amplification of the intrinsic and extrinsic pathway of coagulation cascade by binding directly to the catalytic pocket of factor Xa and thereby preventing the formation of thrombus [2]. It has a molecular formula of $C_{19}H_{18}ClN_3O_5S$ and a molecular weight 435.881 g/mol. Its structure is given in figure No.1. Literature survey revealed that studies had been carried out on Rivaroxaban on RP-HPLC, LCMS/MS, HPTLC [3-13]. The focus of present study was to develop and validate a rapid, stable and economic RP-HPLC method for the estimation of Rivaroxaban in bulk and its formulation. In the present study, a new factor RP-HPLC method was developed which shown high reproducibility and sensitivity. The developed method was validated as per ICH guidelines [14].

EXPERIMENTAL SECTION

Materials used

Chemicals:

Rivaroxaban API and tablets were obtained as a gift sample from MSN PVT LMTD. The chemicals acetonitrile, 0.1% Glacial acetic acid, were HPLC grade, Mumbai, India. Milli-Q water was used.

Instrument:
HPLC (schimadzu) with PDA detector was used. LC Solutions software was used.

Methodology
Preparation of 0.1% glacial acetic acid:
In a 100ml of volumetric flask 0.1ml of Glacial acetic acid (GAA) solution is taken and to this 100ml of milli-Q water was added and then final volume was made up to 100 ml with milli-Q water.
Preparation of Mobile Phase:
An accurately measured 0.1% GAA and Acetonitrile in ratio of 70:30 % v/v were filtered through 0.45µ filter. Acetonitrile was used as a diluent.
Preparation of Standard Solution:
10mg of Rivaroxaban was weighed and placed into a 10 ml of volumetric flask, to this add 5 ml of diluent for 30 minutes, it is sonicated and make up the solution to 10 ml with diluents. From the above stock solution, 1 ml is taken in to a 10ml volumetric flask and make up the solution to final volume with diluent. 1ml is taken in to a 10ml volumetric flask and make up the solution to final volume with diluent.
Preparation of Solution for Selection of Wavelength:
Standard solution of Rivaroxaban was prepared and scanned in the range of 200 nm to 400 nm using a photodiode array detector. The spectrum was recorded.
Analysis of Formulation:
10 tablets were weighed. The Average weight of each tablet was caluculated. Now transfer in to 10ml flask i.e., weight equivalent to 10mg is transferred to flask. To this add 5ml of diluent for 30 min it is sonicated, then final volume was made up with diluent. Then the above solutions was filtered and take 1ml of the filtered solution in to 10ml 0f flask and make up volume with 10ml of diluent.

RESULTS AND DISCUSSION

Validation of developed method
Method validation as per International Conference of Harmonization is defined as "establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics".

System Suitability Testing
The chromatographic conditions for the estimation of Rivaraxoban were discussed in Table 1. Rivaroxaban standard drug solution was injected into HPLC system for six times, and checked for the system suitability parameters like theoretical plates, tailing factor and % RSD of areas for six injections of standard Rivaroxaban drug solution was calculated. The results were shown in the Table 2.

Accuracy
The accuracy of the method was determined by standard addition method. Known amount of standard drug was added to pre analyzed sample of Rivaroxaban in according to 80%, 100% and 120% levels of labelled claim and then subjected to the proposed method. The percentage recovery was calculated and results are presented in Table 3. Satisfactory recoveries ranging from 98% to 102% were obtained by the proposed method. This indicates that the proposed method was accurate.

Precision
Precision of the method was studied by carrying out intraday, inter day analysis and expressed as percentage Relative Standard Deviation. For this purpose 6µg/ml solution was prepared and their peak area of the solutions were measured for six times within the same day and in different days at 250nm results are presented in Table 4 & 5.

Linearity
It is the ability of the method to elicit test results directly proportional to analyte concentration within a given range. Linearity was performed by preparing standard solutions of Rivaroxaban at different concentration levels 2, 4, 6, 8, 10µg/ml and the peak responses were read at 250nm and the corresponding chromatograms were recorded A linearity plot of concentration over peak areas was constructed. The results were presented in Table 6.

Limit of Detection (LOD) and Limit of Quantization (LOQ)

LOD and LOQ of the drug were calculated using the following equations according to International Conference on Harmonization (ICH) guidelines

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where σ = the standard deviation of the response and

S = the slope of the regression equation.

Robustness

Deliberate variations were made to the optimized HPLC conditions, to evaluate robustness, variations made were, flow rate varied by ±2ml/min, Column oven temperature by ±5°C, wave length varied by ±2nm, mobile phase ratio ±10ml .The results were presented in Table 8.

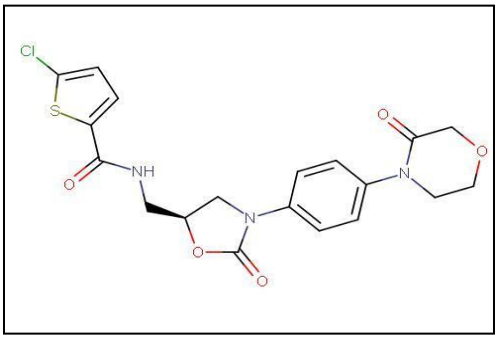

Figure 1: Structure of Rivaroxaban

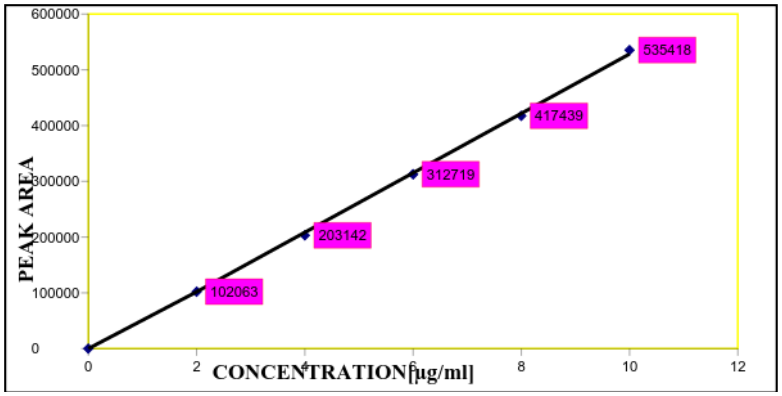

Figure 2: Linearity Curve of Rivaroxaban

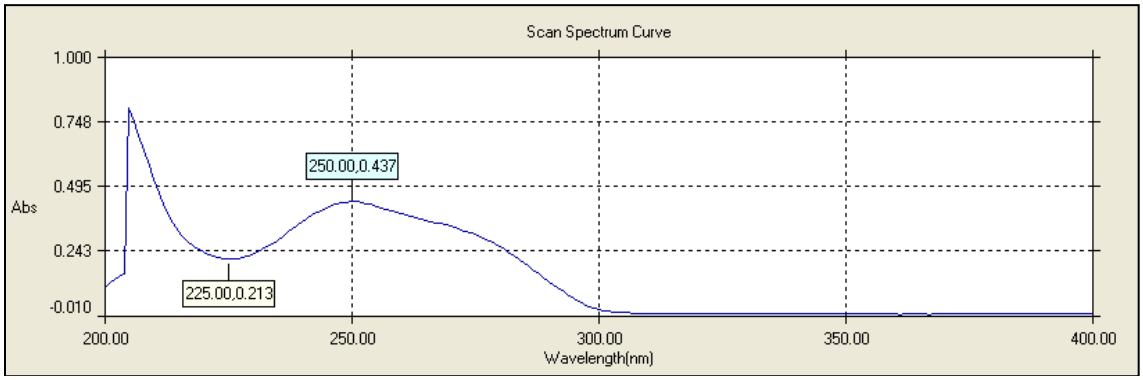

Figure 3: UV spectrum of Rivaroxaban

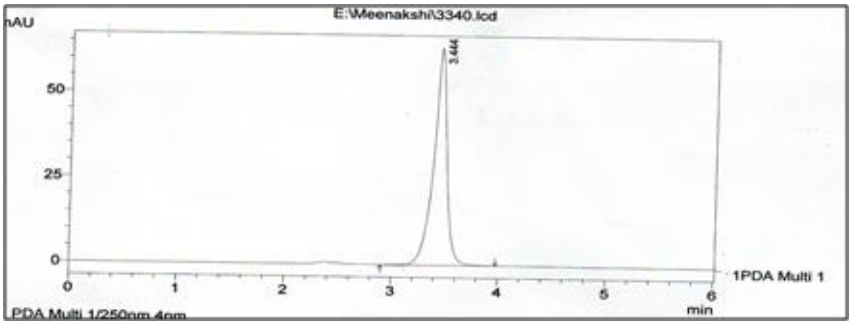

Figure 4: Chromatogram of Rivaroxaban Standard Preparation

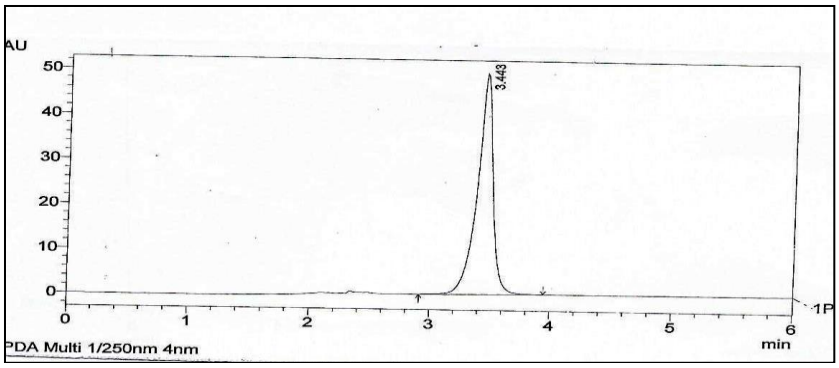

Figure 5: Chromatogram of Rivaroxaban Sample Preparation

Table 1: Optimized chromatographic conditions

S. No	Chromatographic Parameters	Chromatographic Conditions
1	Mode of separation	Binary elution
2	Mobile phase	Acetonitrile : 0.1% GAA (70:30)
3	Column	Enable c18-G (4.6×250mm, 5µm)
4	Flow rate	1.0mL/min
5	Detection Wavelength	250nm
6	Injection Volume	20µl
7	Column oven temperature	Ambient (300c)
8	Run time	6min

Table 2: System Suitability Testing Parameters Results

S. No	System suitability Parameters	Results	Acceptance Criteria
1	Tailing factor	0.905	NMT 2.0%
2	Theoretical plates	4652	NLT 2000
3	% RSD of areas for six injections of Standard Solution.	0.098	NMT 2.0%

Table 3: Results for Accuracy of Rivaroxaban

S. No	% Spike Level	Amount (µg/m)	Amount added (µg/ml)	Amount found (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean % Recovery	SD	% RSD
1	80%	5.99	4.8	10.78	4.79	99.79	99.58	0.21	0.21
2				10.77	4.78	99.58			
3				10.76	4.77	99.37			
4	100%		6	11.94	5.95	99.1	99.23	0.32	0.32
5				11.93	5.94	99			
6				11.97	5.98	99.6			
7	120%		7.2	13.16	7.17	99.5	100	0.55	0.55
8				13.24	7.25	100.6			
9				13.2	7.21	100.1			

Table 4: Intraday precision

S. No	Amount (µg/ml)	Amount found (µg/ml)	Percentage %	% Mean	SD **	% RSD
1	6.0	6.011	100.1	100.1	0.021	0.354
2		6.04	100.4			
3		5.99	99.8			
4		6.03	100.3			
5		6	100			
6		6.04	100.4			

** average of six determinations

Table 5: Inter day precision

S. No	Amount (µg/ml)	Amount found (µg/ml)	Percentage %	Mean	SD*	% RSD
1	6 µg/ml	6	100	100.11	0.525	0.08
2		6.06	100.6			
3		6.02	100.2			
4		6.08	100.8			
5		6.03	100.3			
6		5.93	98.8			

Table 6: Linearity Results

Linearity level	Concentration (µg/ml)	Area			Mean
		Set-1	Set-2	Set-3	
I	2	100642	102241	103307	102063
II	4	204958	200432	204037	203142
III	6	310893	313632	313632	312719
IV	8	419110	415812	417397	417439
V	10	539857	535121	531278	535418
Y = 54050X-10146 R2= 0.9993					

Table 7: Assay of Formulation

S. No	Label claim	Amount Found(n=5)	Assay	% RSD
1	10mg	9.99 mg	99.90%	0.15

Table 8: Robustness Results

Parameter	Normal	Variation	Rt	Tailing factor	Theoretical plates	% RSD
Wave length variation	250	248	3.439	0.891	4563	0.122
		252	3.45	0.922	4732	0.173
Flow Rate variation	1	0.8	4.336	0.894	4971	0.389
		1.2	2.9	0.896	4213	0.139
Column oven Temperature variation	30˚c	25˚c	3.472	0.904	4734	0.04
		35˚c	3.441	0.898	4527	0.183
Mobile phase variation	70:30:00	60:40:00	3.91	0.989	4313	0.134
		80:20:00	3.401	0.997	4282	0.164

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