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Quality by Design Guided Green Rp-Hplc Method Development and Validation for Estimation of Rivaroxaban in Tablet Dosage Form

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ABSTRACT:
A robust, eco-friendly, rapid, and reproducible Quality by Design (QbD)-assisted Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method was developed and validated for quantitative estimation of Rivaroxaban in tablet dosage form. Method optimization was performed using Design Expert® software employing an optimal factorial design approach. Chromatographic separation was achieved using a Kromasil C18 column (250 mm × 4.6 mm, 5 µm particle size) with a mobile phase consisting of acetonitrile and water adjusted to pH 4.5 in the ratio of 60:40 v/v at a flow rate of 1.0 mL/min. Detection was carried out at 250 nm using UV detector. The retention time for Rivaroxaban was observed at 2.582 min. The developed method exhibited excellent linearity over the concentration range of 5–30 µg/mL with correlation coefficient (R^2) of 0.9998. Precision studies showed %RSD below 2%, indicating good reproducibility of the method. Accuracy studies demonstrated recovery in the range of 99.92–100.36%. The developed method was found robust against deliberate changes in chromatographic parameters such as mobile phase composition, wavelength, pH, and flow rate. The assay value of marketed formulation was found within acceptable limits (98–102%). Validation parameters were evaluated according to ICH Q2(R1) guidelines. The proposed analytical method can be successfully employed for routine quality control analysis of Rivaroxaban in pharmaceutical dosage forms.

1. INTRODUCTION:
Rivaroxaban is chemically designated as (S)-5-chloro-N-{[2-oxo-3-[4-(3-oxomorpholin-4-yl) phenyl] oxazolidin-5-yl] methyl} thiophene-2-carboxamide and belongs to the class of direct Factor Xa inhibitors. It is widely

prescribed for the treatment and prevention of thromboembolic disorders including deep vein thrombosis (DVT), pulmonary embolism (PE), and stroke prevention associated with atrial fibrillation. Compared to conventional anticoagulants such as warfarin, Rivaroxaban offers advantages including predictable pharmacokinetics, fewer food interactions, and elimination of routine INR monitoring.

Analytical method development for Rivaroxaban requires sensitive, selective, and reproducible chromatographic conditions suitable for routine quality control applications. Conventional method development approaches are time consuming and require extensive experimentation. Therefore, implementation of Quality by Design (QbD) principles in analytical method development has gained considerable importance in pharmaceutical industries. QbD provides systematic understanding of method variables and their influence on analytical responses, resulting in robust and reliable analytical methods.

Green analytical chemistry is another important aspect in modern pharmaceutical analysis. Reduction in solvent consumption, shorter run time, and minimal generation of hazardous waste are considered critical factors during analytical method development. In the present study, an environmentally conscious RP-HPLC method was developed using optimized chromatographic conditions with reduced analysis time.

The present work focuses on development and validation of a QbD-guided RP-HPLC analytical method for estimation of Rivaroxaban in tablet dosage form according to International Council for Harmonisation (ICH) Q2(R1) guidelines.

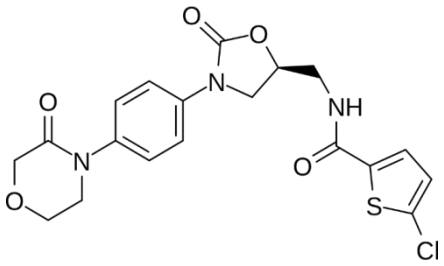

Fig.1. Structure of Rivaroxaban

2. MATERIALS AND METHODS:

2.1 Chemicals and Reagents

Rivaroxaban API was obtained as a gift sample from GHPL, Pune, India. Marketed formulation of Rivaban 10 tablets containing 10 mg Rivaroxaban was procured from local pharmacy. HPLC grade acetonitrile and methanol were used throughout the study. Potassium dihydrogen phosphate, triethylamine, sodium hydroxide, and distilled water were of analytical grade.

2.2 Instrumentation

Chromatographic analysis was performed using Shimadzu LC-2030 HPLC system equipped with UV detector and Lab Solutions software. Separation was achieved on Kromasil C18 column (250 mm × 4.6 mm, 5 µm). Additional instruments used included analytical balance (Mettler Toledo), sonicator (Labman), and pH meter (Lab India).

2.3 Preliminary Characterization of Drug

2.3.1 Description

The physical characteristics including color and appearance of Rivaroxaban were evaluated and compared with reported literature.

2.3.2 Solubility Study

Solubility of Rivaroxaban was studied in various solvents such as methanol, ethanol, acetonitrile, water and dimethyl sulfoxide (DMSO).

2.3.3 UV Spectroscopic Analysis

Standard drug solution was scanned in the range of 200–400 nm using UV spectrophotometer. Maximum

absorbance was observed at 250nm, which was selected for further chromatographic analysis.

3. QUALITY BY DESIGN APPROACH:

3.1 Experimental Design:

A QbD-based optimal factorial design was employed using Design Expert® software for method optimization.
Critical method parameters (CMPs) selected were: Mobile phase composition, pH of aqueous phase.
Critical analytical attributes (CAAs) selected were: Retention time, tailing factor, theoretical plates and peak area.
The experimental ranges investigated were: Organic phase composition: 40–60%, pH range: 3.5–4.5.
Nine experimental runs were suggested by the software for optimization studies.

3.2 Preparation of Mobile Phase

The aqueous phase was prepared by adjusting water to pH 4.5 using dilute triethylamine. The optimized mobile phase consisted of acetonitrile and water (60:40 v/v). The mobile phase was filtered through 0.45 µm membrane filter and degassed prior to use.

3.3 Preparation of Standard Solution

Accurately weighed 10 mg of Rivaroxaban was dissolved in methanol and diluted up to 10 mL to obtain stock solution of 1000 µg/mL. Further dilution was carried out to obtain working standard solution of 10 µg/mL.

4. OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS

Among the tested conditions, acetonitrile-water combination showed better peak symmetry, acceptable retention time, and improved theoretical plates.

Table 1. The optimized chromatographic conditions were:

Parameter	Optimized Condition
Column	Kromasil C18 (250mm × 4.6 mm, 5 µm)
Mobile phase	Acetonitrile:Water (60:40 v/v)
pH	4.5
Flow rate	1.0 mL/min
Detection wavelength	250 nm
Injection volume	10 µL
Run time	6 min
Retention time	2.582 min
Temperature	Ambient

4.1 Effect of Independent Variables

4.1.1 Effect on Retention Time

Increase in acetonitrile concentration resulted in decreased retention time. pH variation showed comparatively lesser influence on retention behavior.

4.1.2 Effect on Tailing Factor

Increase in organic phase concentration and slight increase in pH improved peak symmetry and reduced tailing factor.

4.1.3 Effect on Theoretical Plates

Increase in acetonitrile concentration significantly improved column efficiency and theoretical plate count.
The optimization process confirmed that the selected chromatographic conditions provided desirable analytical performance with excellent peak characteristics.

5. METHOD VALIDATION

Method validation was performed according to ICH Q2(R1) guidelines.

5.1 Linearity

The developed method exhibited linearity in concentration range of 5–30 µg/mL.

Table 2: Linearity Result of Rivaroxaban.

Conc. (µg/mL)	Peak Area
5	769225
10	1538450
15	2257675
20	3076900
25	3846125
30	4615350

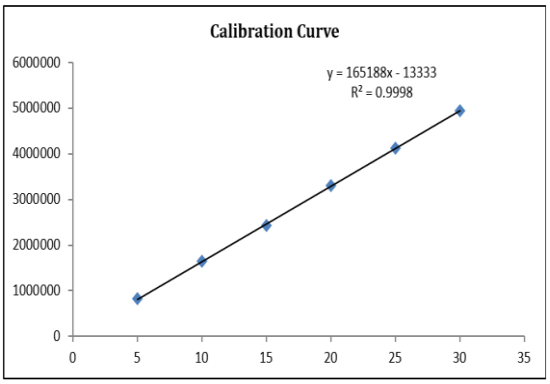

Fig.2. Calibration curve of Rivaroxaban

5.2 System Suitability

System suitability parameters were evaluated using six replicate injections.

Table 3: System Suitability Studies of Rivaroxaban by HPLC Method

Parameter	Result
Retention time	2.582 min
Theoretical plates	6993
Tailing factor	0.8
Peak area	769225

All parameters were found within acceptable limits.

5.3 Specificity

The developed method was found specific for Rivaroxaban with no interference from blank or excipients at retention time of analyte.

Table 4: Specificity of Rivaroxaban by HPLC Method

Sample	Label Claim (mg)	Amount Found	Recovery (%)	Retention Time (min)
Tablet	10	9.91	99.1	2.582

5.4 Precision

Precision studies were performed as repeatability precision.

Table 5: Precision.

Precision		
Repeatability		
Sr. No	**Concentration (µg/mL)**	**Peak Area**
1	20	3082118
2	20	3062118
3	20	3082131
4	20	3075244
5	20	3132118
6	20	3079906
Average		3085605.8
Standard Deviation		21905.7
RSD%		0.710

The %RSD value below 2% indicated good precision.

5.5 Accuracy

Accuracy was evaluated using recovery studies at 80%, 100%, and 120% concentration levels.

Table 6: Accuracy of Rivaroxaban at 250 nm.

Recovery				
Sr. No	**Amount of Sample**	**Amount of Drug Added**	**Amount of Drug Recovered**	**Recovery %**
	(µg/ml)	(µg/ml)	(µg/ml)	
1	16	2449401.6	15.99	99.92
2	20	3061752	19.99	99.95
3	24	3674102.4	24.09	100.36

The obtained recovery values confirmed accuracy of the developed method.

5.6 Sensitivity:

The LOD and LOQ were calculated by the use of signal to noise ratio. The Limit of Detection (LOD) was 0.4844 µg/mL & The Limit of Quantification (LOQ) was 1.4679 µg/mL.

5.7 Robustness

Robustness studies were performed by deliberate variation in chromatographic conditions such as flow rate, wavelength, pH, and mobile phase composition.

Table 7: Robustness of Rivaroxaban

Robustness					
Sr. No	**Parameter**		**Response**	**Parameter**	**Response**
Acetonitrile: Water			**Retention Time (min)**	**Detection Wavelength**	**Peak Area**
(V/V)				(nm)	
1	59	41	2.485	248	3018773
2	60	40	2.582	250	3077088
3	61	39	2.681	252	3110352
Average			2.583	Average	3068738
Standard Deviation			0.080	Standard Deviation	37850.36
RSD%			3.098	**RSD%**	1.233
Flow Rate			**Retention Time (min)**	**pH of Buffer**	**Peak Area**
(mL/min)				(mmol/L)	
1	0.9		2.713	4.3	3105160
2	1		2.582	4.5	3077261
3	1.1		2.5779	4.7	3001640
Average			2.624	Average	3061354
Standard Deviation			0.0627	Standard Deviation	43733.13
RSD%			2.391	**RSD%**	1.4286

The developed method was found robust under varied analytical conditions.

6.RESULTS AND DISCUSSION:

A simple, rapid, accurate, and eco-friendly RP-HPLC analytical method was successfully developed for quantitative estimation of Rivaroxaban in tablet dosage form using QbD principles. The application of experimental design enabled systematic optimization of chromatographic conditions while minimizing the number of experimental trials.

The developed method demonstrated excellent chromatographic separation with retention time of 2.582 min and acceptable system suitability parameters. The shorter run time and reduced solvent consumption support the green analytical aspect of the proposed method.

Validation results confirmed that the developed method is linear, accurate, precise, specific, and robust according to ICH Q2(R1) guidelines. The proposed method can therefore be effectively employed for routine quality control analysis of Rivaroxaban in pharmaceutical formulations.

7. CONCLUSION:

The present study successfully established a Quality by Design assisted RP-HPLC analytical method for estimation of Rivaroxaban in tablet dosage form. The developed method demonstrated excellent analytical performance with high sensitivity, accuracy, precision, and robustness. The QbD approach enabled systematic optimization of chromatographic parameters and improved method understanding. The proposed method also fulfilled the principles of green analytical chemistry due to reduced solvent consumption and shorter analysis time.

The validation studies performed according to ICH guidelines confirmed suitability of the developed method for routine pharmaceutical analysis and quality control applications. Hence, the proposed RP-HPLC method can be effectively utilized for regular assay determination of Rivaroxaban in bulk and marketed formulations.

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CONFLICT OF INTEREST:
The authors declare that there is no conflict of interest regarding publication of this manuscript.

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