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Development and validation of stability indicating assay by HPLC method for estimation of Rivaroxaban

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Abstract: A novel high speed, high resolution Reverse phase-HPLC method was developed for estimation of assay in Rivaroxaban drug substance. The separation of drug from the possible impurities was achieved in an Inertsil C8 column. Potassium phosphate buffer at pH 3.0 and acetonitrile mixture was selected as mobile phase. Flow rate and detection were kept at 1.0 mL/min and 250 nm respectively. Column compartment temperature was maintained at 40 °c and sample compartment temperature was kept at ambient. Mixture of buffer with acetonitrile was selected as sample diluent. The developed HPLC method was subjected to validation parameters; Precision, Specificity, Linearity, Robustness, Ruggedness were established as per the guidelines recommended by ICH. Stability indicating nature of the method was also performed by exposing the sample under various conditions like acid, base, peroxide and photo stability conditions. Using the method one can carry out the quantitative estimation of assay in Rivaroxaban drug substance, further the same method can be adopted for determination of related substances also.

Key words: Rivaroxaban, Anticoagulant, High Performance Liquid Chromatography, Forced degradation

Introduction

Rivaroxaban is chemically 5-Chloro-N-({(5S)-2-oxo-3-[4-(3-oxo-4-morpholinyl) phenyl]-1,3-oxazolidin-5yl} methyl)-2-thiophenecarboxamide. The empirical formula is $C_{19}H_{18}ClN_3O_5S$ and the molecular weight is 435.89 g/mol. Rivaroxaban is a pure (S)-enantiomer. It is an odorless, non-hygroscopic and white to yellowish powder. Rivaroxaban is only slightly soluble in organic solvents and is practically insoluble in water and aqueous media. It is sold under the brand name of Xarelto contains 2.5 mg, 10 mg and 15 mg or 20 mg of Rivaroxaban drug substance. Rivaroxaban is a anticoagulant which is used for prevention of stroke and venous embolism in patients with chronic atrial fibrillation [1, 2]

As per the literature, rivaroxaban is a non compendia product, determination of rivaroxaban in pharmaceutical drug substance and dosage forms by spectro photometric method [3, 4 and 5] and LC-MS/MS [6, 7] methods. LC [8, 9 and10] assay and related substances methods were proposed for drug substance and formulated evaluation. Since the drug is being available in domestic and international market the aim of investigation by the author was to develop a rapid, accurate and precise RP-HPLC methodfor the determination of assay. The developed method was subjected to validation parameters [11, 12] such as precision, linearity, accuracy, robustness and ruggedness to prove accurate, precise and rugged method. The method was validated according to ICH requirements [13, 14 and 15]. By exposing the drug to various stress conditions like acid hydrolysis, Base hydrolysis, heat and photo stability, stability indicating nature of the method was assessed.

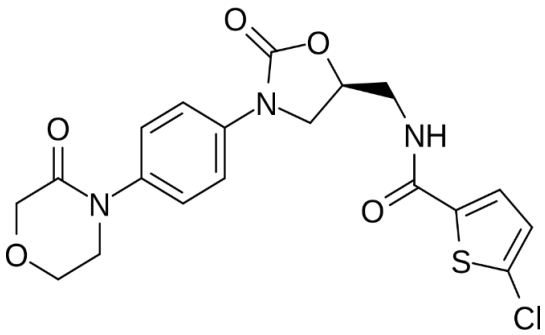

Figure 1. Chemical structure of Rivaroxaban

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Materials and Methods

Instrumentation and Reagents

High performance liquid chromatograph manufactured by Agilent with photo diode array configuration was used for the present study. Merck AR Grade potassium dihydrogen phosphate and Gradient grade acetonitrile reagents were used to prepare mobile phase. The Hplc column adopted for the study was Inertsil C8-3, 250 x 4.6 mm 5µm, from GL sciences.

Chromatographic Conditions

Separation of possible impurities was achieved on a Inertsil C8-3, 250 x 4.6 mm 5µm Column. Mobile phase consists of 20 mM Potassium dihydrogen phosphate and HPLC grade acetonitrile. Mobile phase flow rate kept at 1.0mL/min with a simple gradient. Gradient program was set as Time/ % of solution B: 0/25, 5/25, 25/35, 25.5/25. Column temperature was maintained at 40°c and detection was carried at 250 nm. Temperature of sample compartment was maintained at ambient and the volume injected with auto injector was 5µL. Buffer with acetonitrile in the ratio of 1:1 was selected as diluent for preparation of standard and test solutions.

Preparation of Standard and Sample

Accurately weighed and transferred 25 mg of Standard solution and test solutions in a 50 mL volumetric flask separately, dissolve and dilute to the volume with diluent. Final concentration of Rivaroxaban in the sample and standard was 0.5 mg/mL.

Method Validation

System suitability

A system suitability solution shall be injected to ensure the readiness of system before analysis, a standard solutionis injected on to the system and verified Tailing factor (T), column efficiency (N) of drug substance.

Specificity

Specificity is the ability of method to measure the analyte response in the presence of its potential impurities. The specificity of the developed RP-HPLC method is demonstrated by forced degradation studies to prove stability indicating nature.

Precision

Precision of the method shall be reported by injecting six replicates of standard solution consecutively under the same analytical conditions. Percentage relative standard deviation for six replicates should be calculated.

Linearity

Linearity of assay method shall be demonstrated with five different concentration levels. The solutions are prepared at five different concentration levels ranging from 60% to 140%. The calibration curve is to be drawn by plotting peak areas of drug substance and its corresponding concentrations. Correlation coefficients, slope of the curve are reported to show the developed method was linear.

Accuracy:

Test solutions of Rivaroxaban shall be prepared at three concentration levels corresponding to 60%, 100% and 140%. The % recovery of three levels was reported against standard concentration. Calculation of % Recovery is based on amount of test solution added vs. standard concentration. Percentage recovery value should be not less than 80% and not more than 120%.

Results and Discussions

Method Development and optimization

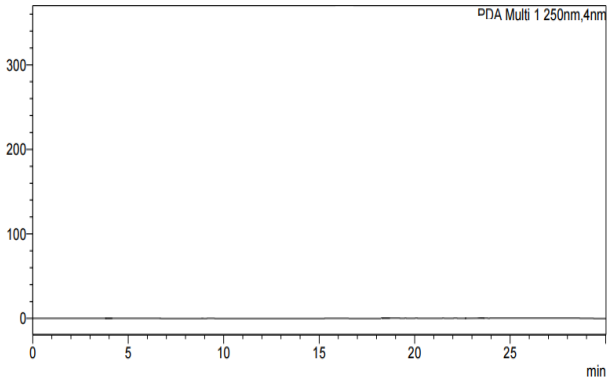
The objective of the development is to have more specific, precise, robust and accurate method to estimate content of drug present in drug substance and drug product to support routine quality check at competitive time period. Selected wavelength for the detection of drug substances was 250nm, a simple gradient program was applied to resolve all degradants and to eliminate interference with diluent and unidentified peaks from sample.

System Suitability Results

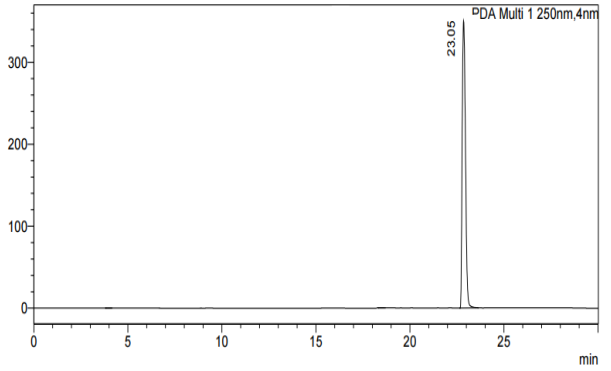
The symmetry, Plate count and homogeneity of Rivaroxaban drug substance was found optimal with the selected chromatographic conditions. A typical system suitability chromatogram of sample diluent, standard solution and test solution chromatograms are shown in Figure 2(a), 2(b) and 2(c). In the optimized of more than 5000 shows the efficiency of column. System suitability parameters for Rivaroxaban drug substance are tabulated in Table 1.

Table 1: System Suitability Results

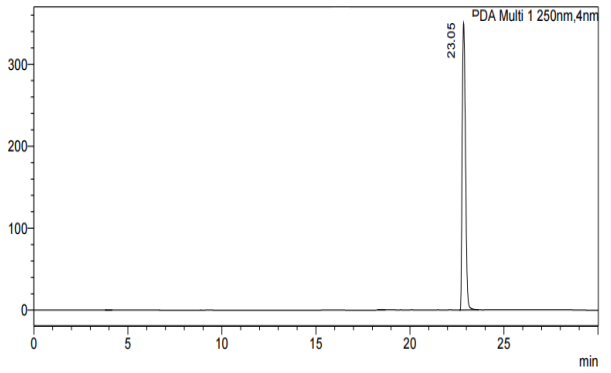
Component	RT	Tailing factor	Plate count	Peak angle	Peak threshold
Rivaroxaban	23.0	1.1	8200	4.156	5.250



a)Sample diluent



b) Standard solution



c)Test solution

Figure 2: A typical chromatogram of Rivaroxaban (a) sample diluent (b) Standard solution (c) Test solution

Method Validation Results

Precision

System precision was evaluated by performing five replicates of standard solution at the specified concentration level. The % relative standard deviation of 5 injections was within the acceptable limit. The obtained % relative standard deviation was less than 1.0%, which indicates the system is highly precise to proceed for analysis. Results are tabulated in Table 2.

Table 2. System precision results

System precision	
No. of Injections	**Peak area**
1	5371689
2	5299884
3	5345996
4	5289564
5	5402255
AVG	**5341878**
STD DEV	47569.12
%RSD	**0.89**

Linearity

Linearity of the method is to establish a linear relationship of concentration against response. Solutions of Rivaroxaban were prepared in the range of 60% to 140% of the standard concentration level. Correlation coefficient obtained was greater than 0.99. The regression statistics for Rivaroxaban drug substance are tabulated in Table-3. Linearity plot is shown in Figure-3. The result shows that an excellent correlation between the peak response and concentration of analyte.

Table 3: Linearity Results

Linearity		
Standard weight		25.25mg
Potency of standard		0.9922
No.Of Inj	**Conc (PPM)**	**Response**
1	300.00	3194569
2	400.00	4197556
3	500.00	5371659
4	600.00	6394589
5	700.00	7242032
Slope		**10291.96**
Y-intercept		**134101.50**
Correlation coeffficient		**0.9987**

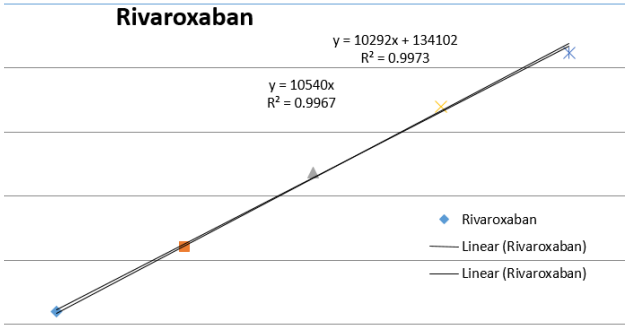


Figure 3. Linearity plot of Rivaroxaban

Accuracy

Accuracy of the assay method can be determined by comparing three different test concentration levels with standard solution concentration. The obtained recovery value indicates the trueness of the method to estimate assay of drug substance.

Rivaroxaban test solution was prepared in a concentration range varying from 60% to 140% of the target analyte concentration. The acceptance criterion for accuracy is 80% to 120%. The obtained percentage recovery value is in the range of 97.5% to 99.5% which declares the method accuracy. Accuracy results are reported in Table 5.

Table 4: Accuracy Results

S.NO	Accuracy Level	Dabigatran recovery	Criteria
1	60%	97.5%	80% to 120%
2	100%	99.4%	
3	140%	99.5%	

Forced degradation study

Degradation studies were performed to demonstrate stability indicating nature of the method. Rivaroxaban test sample was exposed to various stress conditions like heat & humidity (40°C & 70% RH for 7 days), thermal (60°C for 7 days) and photolytic conditions of fluorescent light (1.2x106 LUX hours), UV light for a total exposure of 200 W·hr/m2, acid hydrolysis (0.1N HCl 70°C for 2 Hrs), base hydrolysis (0.1N NaOH, RT for 1 Hrs) and oxidative stress (3% Peroxide at 70°C for 48 hrs). Testing peak purity gives homogeneity.

Peak obtained in all the stress conditions was homogenous and unaffected by the presence of its degradation impurities, confirming the stability indicating nature of the method. Mass balance also established to match up the sum of impurities with its assay value against reference unstressed sample. The results from forced degradation studies are summarized in Table 5.

Table5: Forced degradation studies

Stress condition	Conc. µg/mL	% Degradation found	Purity angle	Purity Threshold	Mass balance
Non stressed	500.25	99.9	1.24	2.45	100.0
Acid hydrolysis	500.90	93.5	1.96	3.24	99.6
Base hydrolysis	501.05	85.6	1.55	2.66	99.1
Oxidation	500.80	99.5	1.44	2.22	99.8
Heat and humidity	500.95	99.4	1.11	2.33	99.3
Photo stability	500.15	99.1	1.99	2.45	98.9
Dry heat	500.55	99.5	1.65	2.56	98.8

Conclusion

A Reverse phase-HPLC method was proposed for the estimation of assay in Rivaroxaban drug substance. The developed method was subjected to method validation parameters as recommended by ICH. Stability indicating nature of the method was demonstrated by applying forced degradation studies. The developed method was specific, precise, accurate and linear to estimate accurate amount of drug present in the sample. Degradation studies confirmed the homogeneity and free of interferences with the peak of interest. The method can be adopted to determine the assay of drug substance in quality control labs.

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