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ANALYTICAL METHOD VALIDATION REPORT OF RIVAROXABAN ASSAY BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract: A simple, accurate, rapid and precise high-performance liquid chromatographic method has been developed and validated for Rivaroxaban in bulk. The chromatographic validation was carried out on Atlantis C-18 analytical column (4.6×250 mm; 5µm) with a mixture of Buffer solution (Weight about 1.36gm KH_2PO_4 in 1000mL of Milli-Q Water and adjust its pH to 5.0(± 0.05) with diluted KOH (0.1%w/v)) and Acetonitrile as a mobile phase; at a flow rate of 1.0 mL/min. UV detection was performed at 248 nm. The retention times were 16.732 min. Calibration plot was linear (r^2=0.9998) over the concentration range of 75-225 µg/mL. The method was validated for accuracy, precision, specificity, linearity, and sensitivity. The proposed method was successfully used for quantitative analysis of tablets.

Keywords: Rivaroxaban, HPLC, Validation and Quantitative analysis.

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INTRODUCTION

Rivaroxaban (5-chloro-*N*-({(5S)-2-oxo-3-[4-(3-oxo-4-morpholinyl) phenyl]-1,3-oxazolidin-5-yl}methyl)-2-thiophenecarboxamide)[1]. Is a novel, oral, selective, highly potent, direct FXa inhibitor. In preclinical studies, rivaroxaban demonstrated consistent and potent anticoagulant and antithrombotic effects [2, 3]. Furthermore, the efficacy and safety of rivaroxaban have been demonstrated in clinical phase I–III trials [4–10]. Through serve of literature reveals that there are a few analytical methods have been developed for the determination of Rivaroxaban *viz,*. High-performance liquid chromatography–tandem mass spectrometry [11-12], bioanalytical assay validation [13, 14].

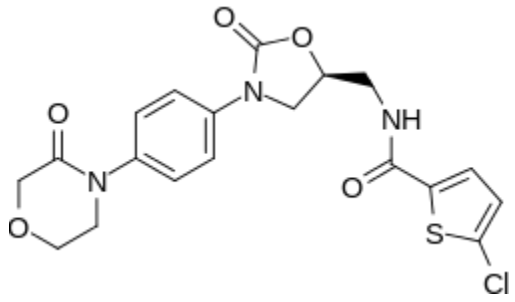

Fig (1): Rivaroxaban

ANALYTICAL PROCEDURE

Chromatographic conditions:

Standards used for validation were RVBKSM-III (RS/RVB/008) and RIVAROXABAN (RS/RVB/010). The chromatographic validation was carried out by using Waters e2695 HPLC Instrument on Atlantis C-18 analytical column (4.6×250 mm; 5µm) with a mixture of Buffer solution (Weight about 1.36gm KH_2PO_4 in 1000mL of Milli-Q Water and adjust its pH to 5.0(± 0.05) with diluted KOH (0.1%w/v)) and Acetonitrile as a mobile phase; at a flow rate of 1.0 mL/min. UV detection was performed at 248 nm. All chemicals and reagents used were HPLC grade and purchased from Merck chemicals, India.

Diluent: Acetonitrile: Methanol: water (25:45:30v/v/v) and run time is 55min.

Preparation of Standard Solution:

Weighed accurately about 15 mg of RVB standard in 100 ml volumetric flask, added diluent, shake and sonicated well to dissolve and diluted up to the mark with diluents (150µg/ml).

Preparation of Sample Solution:

Weighed accurately about 15 mg of sample in 100 ml volumetric flask, added diluent, shake and sonicated well to dissolve and diluted up to the mark with diluents (150µg/ml).

Calculation:

$$\text{Assay \%w/w} = \frac{\text{SPL (Area)}}{\text{STD (Area)}} \times \frac{\text{Conc. STD}}{\text{Conc. SPL}} \times P \times \frac{100}{(100\text{-LOD})}$$

Where:

SPL (Area) – is area of peak of RVB in sample solution.

STD (Area) is Mean area peak of RVB from five injections of standard solutions.

Conc. STD – Concentration of RVB from standard solution in mg/ml.

Conc.SPL – Concentration of sample solution in mg/ml.

P – Potency of RVB standard in %.

LOD – Loss on drying in %.

VALIDATION OF ASSAY METHOD

Specificity: Specificity is defined as, ability of analytical method to assess unequivocally the analyte in the presence of components that may be expected to present, such as impurities, degradation products.

By injecting diluents & individual components into the chromatograph.

Diluent, Rivaroxaban Stage I, Rivaroxaban stage II, Rivaroxaban Stage III, Rivaroxaban, Rivaroxaban KSM I, Rivaroxaban KSM II & Rivaroxaban KSM III injected individually into the chromatograph. Retention time of all the components is noted.

Component	Retention time	RRT
RVB KSM - I	3.865	0.24
RVB KSM - II	12.657	0.78
RVB KSM - III	4.814	0.30
RVB STAG -I	11.356	0.70
RVB STAGE - II	14.025	0.87
RVB STAGE - III	2.475	0.15
RIVAROXABAN	16.149	1.0

It is observed from the above table that Rivaroxaban Stage I, Rivaroxaban stage II, Rivaroxaban Stage III, Rivaroxaban, Rivaroxaban KSM I, Rivaroxaban KSM II & Rivaroxaban KSM III were well resolved from each other.

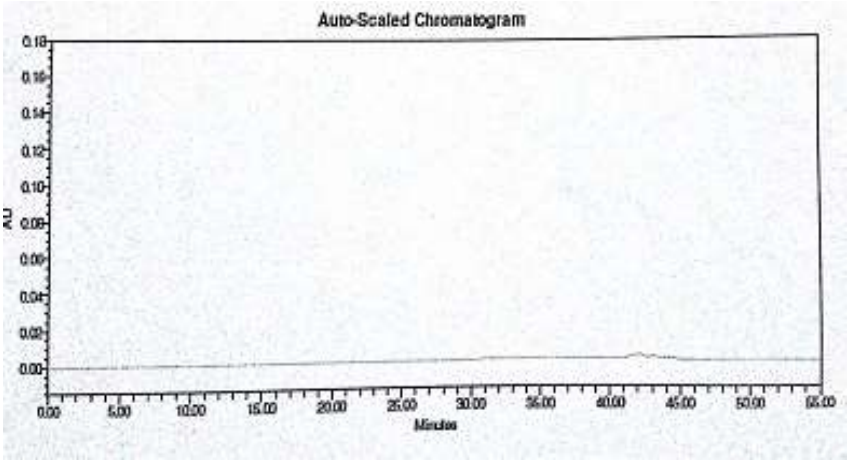

Fig (2): Chromatogram of Blank

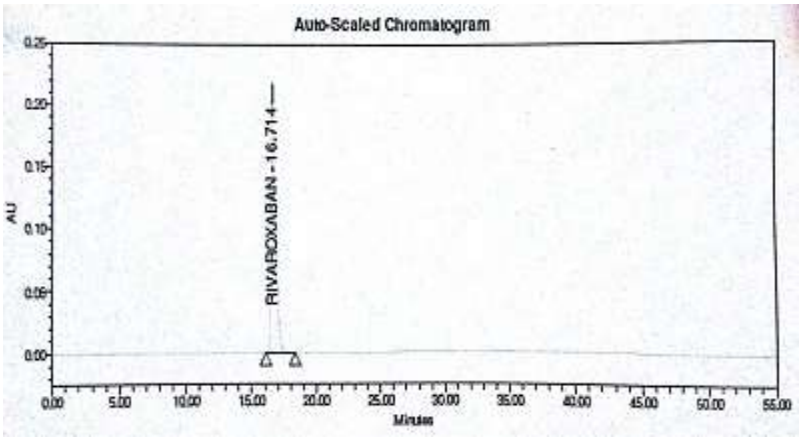

Fig (3): Chromatogram of Standard solution

By forced degradation study:

Forced degradation study is carried out to know in advance likely degradation products that may be generated during stability study or shelf life. Rivaroxaban is exposed to the following dry environmental and wet chemical conditions.

A. Dry environmental conditions:

1. White fluorescent lamp (NLT 1.2 million lux hrs.)

2. UV Radiation (NLT 200 watt hrs/ square metre.)

3. Heat (at 80^0c for 8 hrs.)

Procedure:

Kept 1 g of sample for above condition. Weighed 75 mg of sample in 100 ml volumetric flask, dissolved and diluted up to the mark with diluents.

B. Wet chemical conditions:

1. Basic (Sodium hydroxide, 1N, at 80^0c for 2 hrs)

2. Acidic (Hydrochloric acid, 1N, at 80^0c for 8 hrs)

3.Oxidation (H_2O_2-3% Heat at 80^0c for 8 hrs)

4. Aqueous Hydrolysis (at 80^0c for 8 hrs)

Procedure:

Weighed 75 mg of sample in 100 ml volumetric flask, added 5ml of respective chemical and kept on water bath at 80^0c for respective time interval. Neutralized the solution if necessary and dilute up to the mark with diluents.

Observation

Sample	Assay	% Impurities	Mass balance	Purity angle	Purity Threshold
Sample as such	99.2	0.04	99.2	0.204	0.319
Sample exposed to 1N HCL 8hrs – 80^0c	95.5	2.640	98.1	0.172	0.294
Sample exposed to 1N NaOH 2hrs -80^0c	89.5	10.38	99.9	0.171	0.277
Sample exposed to 3% H_2O_2 8hrs – 80^0c	98.2	0.140	98.3	0.193	0.297
Sample exposed to 1.2 million lux hrs	98.5	0.00	98.5	0.230	0.313
Sample exposed to NIT 200 watts/hrs	99.5	0.00	99.5	0.224	0.317
Sample exposed to Thermal degradation 8hrs – 80^0c	99.0	0.04	99.0	0.207	0.312
Sample exposed to Aqueous degradation 8hrs- 60^0c	99.8	0.00	99.8	0.214	0.306

From the above forced degradation table, it is concluded that-

a. In dry environmental conditions, i.e. White fluorescent lamp (NLT 1.2 million lux hrs), UV Radiation (NLT 200 watt hrs / square metre, thermal degradation, no degradation was observed.

b. In alkaline condition, total 10.38% of degradation was observed.

c. In acidic condition, Total 2.64% of degradation was observed.

d. In oxidation condition, Total 0.14% of degradation was observed.

Conclusion:

In all above conditions, where the degradation is observed, main peak of Rivaroxaban is found to be pure and no any other peak is merged in it (as indicated by PDA detector). All degradant are well separated from Rivaroxaban peak indicating specificity and stability indicating nature of the method.

Linearity:

The linearity of an analytical method is its ability to elicit test results that are directly, or by well-defined mathematical transformation, proportion to the concentration of analyte in samples within a given range.

Linearity is determined by serial dilutions of analyte at 5 different concentrations in the range of LOQ to 150% of assay concentration level. Each solution injected into the chromatograph in duplicate and average area of two determinations is recorded.

A graph of concentration (on X axis) VS Area (on Y axis) is plotted. A correlation coefficient, slope and intercept are calculated.

Concentration in (µg/ml)	Peak Area
0.5	12652
1	23064
2	46583
3	70023
4	90598
5	114976
Correlation coefficient	0.9996
Slope	22707
Intercept	990.14

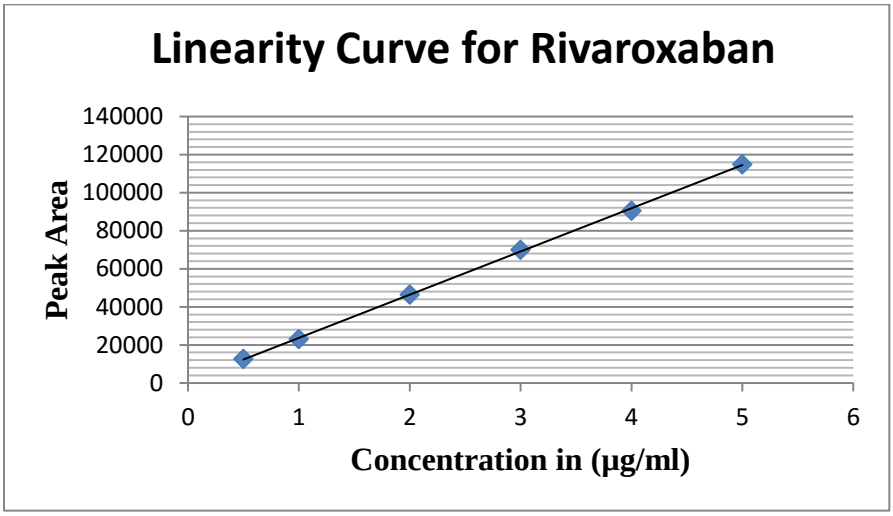

Fig (4): Linearity Curve for Rivaroxaban

Correlation coefficient is found to be more than 0.999 that indicate linearity of the analytical method in specified range.

Precision:

The precision of an analytical method is the degree of agreement among the individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample.

System precision:

To ensure analytical system is working satisfactory and giving precise results, solutions of Rivaroxaban standard at working concentration (150 ppm) was injected into the chromatograph. % RSD for retention time and area is calculated to ensure system suitability and it is tabulated below.

Injection No	RT	AREA
1	16.733	4642475
2	16.742	4623388
3	16.716	4612412
4	16.729	4590895
5	16.729	4572708
6	16.743	4598505
MEAN	16.732	4606731
SD	0.010	24752
RSD	0.06	0.54

The RSD determined peak area is below 1.0% and retention time is below 2.0% respectively indicating analytical system is precise.

Method Precision:

The precision of an analytical method is determined by assaying a sufficient number of aliquots of a homogeneous sample to be able to calculate statistically valid estimates off standard deviation or relative standard deviation. To determine method precision, six samples of pooled sample were prepared at working concentration (150 ppm) and were analyzed by HPCL system. Results found are tabulated below.

Sample No	% Assay
1	99.5
2	99.7
3	99.2
4	99.8
5	99.6
6	99.9
MEAN	99.6
SD	0.25
% RSD	0.25

The RSD value obtained for % Assay found is less than 2.0% indicating analytical method is precise.

Intermediate Precision:

The intermediate precision or ruggedness of the analytical method is the degree of reproducibility of test results obtained by analysis of the same sample under a variety of conditions, such as different analyst, different day, etc. The intermediate precision is performed by repeating same set of experiment done under method precision on different day by different analyst on different instrument. The results obtained from two sets are compared and it is tabulated below.

Analyst II

Sample No	% Assay
1	99.1
2	99.4
3	99.7
4	99.6
5	99.6
6	99.7
MEAN	99.4
SD	0.40
% RSD	0.40

Difference between method precision and intermediate precision study

Parameter	% Assay
Method precision	99.6
Intermediate precision	99.4
Difference	0.20

RSD values obtained for % Assay were below 2.0% for individual sets. Difference between

% Assay for two sets is below 0.5% indicating that analytical method is rugged.

Accuracy:

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy is inferred once precision, linearity and specificity has been established.

Robustness:

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its relativity during normal usage.

Following Chromatographic conditions are changed and sample was analyzed at each condition. The results obtained are then compared with that obtained with standard chromatographic conditions. Variation in the results is determined as a function of relative standard deviation.

Change in pH of buffer solution (±0.1 units)

The Composition is change to ±0.1 units and sample was analyzed. The results obtained are tabulated below.

Parameter	% Assay
pH 4.9	99.7
Method precision	99.6
Difference	0.1
pH 5.1	99.2
Difference	0.4

Change in Column Temperature (± 2^0c):

Column temperature is changed by ± 2^0c and sample is analyzed. The results obtained are tabulated below.

Parameter	% Assay
Temperature 380c	99.8
Method precision	99.6
Difference	0.2
Temperature 420c	100.0
Difference	0.4

Change in Flow (±0.1 mL/min):

The flow of mobile phase is changed by ±0.1 mL/min and sample was analyzed. The results obtained are tabulated below.

Parameter	% Assay
Flow 0.9 mL/min	99.5
Method precision	99.6
Difference	0.1
Flow 1.1 mL/min	99.2
Difference	0.4

STABILITY IN ANALYTICAL SOLUTION:

Stability in analytical solution study is carried out to know the stability of sample in analytical solution (diluents) over a period of time during routine analysis. Sample solution was analyzed

after 0 hrs, 6 hrs, 12 hrs and 24 hrs. Results obtained are the compared with initial results and it is tabulated below.

Interval	% Assay
0 hrs	99.2
6 hrs	99.0
Difference(0 to 6 hrs)	0.2
12 hrs	98.9
Difference(0 to 12 hrs)	0.3
24 hrs	98.8
Difference (0 to 24 hrs)	0.4

From the above data it is evident that the sample at limit level shows no major change in % Assay value at room temperature up to 24 hrs of preparation.

SYSTEM SUITABILITY:

To ensure that the analytical method is working satisfactory and capable to give accurate and precise results, system suitability test has been incorporated in the analytical method. Standard solution was injected into the chromatograph six times to determine the % RSD of area due to Rivaroxaban.

Sr. No	Parameter	Limits	Results
1	% RSD of Rivaroxaaban for Area	NMT 2.0%	0.54
2	% RSD of Rivaroxaaban peak for Retention time	NMT 1.0%	0.06
3	Tailing factor for Rivaroxaban peak	For information	1.35
4	Theoretical plates for Rivaroxaban peak	For information	12967

From above data, it is evident that, all system suitability parameters are within the acceptance a criterion indicting that, the analytical method is working satisfactory.

CONCLUSION

A. Analytical method is found to be specific as proved by injecting known component into the chromatograph.

B. The Analytical method is found to be linear in the specified range.

C. Analytical method is found to be precise and robust.

From the above data, it can be concluded that, the analytical method used for analysis of assay of Rivaroxaban is capable to give accurate and precise results. Hence, it can be used for routine quality control testing.

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