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Development of validated RP- HPLC method for estimation of rivaroxaban in pharmaceutical formulation

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ABSTRACT

A simple RP-HPLC method has been developed and validated for the estimation of Rivaroxaban in formulation. Rivaroxaban is a direct factor Xa inhibitor which is indicated for the prevention various coagulative disorders. A HIBAR-5µ C18 column (250×4.6mm) was used as a stationary phase. The mobile phase consisted of potassium dihydrogen orthophosphate buffer (pH adjusted to 3.0 with orthophosphoric acid): Acetonitrile in the ratio of 60:40 % v/v and the flow rate was 1 ml/min. The detection was carried in the room temperature at 248 nm. The retention time of Rivaroxaban was 7.45 min. The method was linear in the concentration range of 1-5 mcg/ml with correlation coefficient (r^2) of 0.9978. The method was validated as per ICH guideline and it was successfully applied in the estimation of Rivaroxaban in the tablet formulation.

Keywords: RP-HPLC, Rivaroxaban, Potassium dihydrogen orthophosphate buffer.

INTRODUCTION

Rivaroxaban is orally active direct factor Xa inhibitor approved by CDSCO for the prophylaxis treatment of venous thromboembolism[1]. Rivaroxaban is chemically 5-Chloro-N-((5S)-2-oxo-3-[4-(3-oxo-4-morpholinyl) phenyl]-1,3-oxazolidin-5-yl}methyl)-2-thiophene-carboxamide[2]. Rivaroxaban is available in India in the brand name of Xarelto® as a tablet[3]. It is a highly selective direct Factor Xa inhibitor with excellent oral bioavailability and rapid onset of action[4]. There are no official methods for the assay of Rivaroxaban in any pharmacopoeia. The literature review revealed few methods of estimation of Rivaroxaban in its formulation by UV spectroscopy[5-6] and HPLC[7-9]. The present study attempts to develop a simple, rapid and economical RP-HPLC method for the estimation of Rivaroxaban in its tablet form.

MATERIALS AND METHODS

CHEMICALS

All the chemicals used were of analytical grade and the solvents were of HPLC grade which has procured from SDFCL, Mumbai. Rivaroxaban was purchased from Clearsynth Labs, Mumbai. Tablet Xarelto® was procured from local market.

INSTRUMENTS

Shimadzu Prominence UFLC (Shimadzu Corporation, Kyoto, Japan) equipped with LC-20 AD pump, SPD-M20A diode array detector, DGU-20A3 degasar, SK-20

AC auto sampler and CTO- 10 ASVP column oven. Chromatograms were recorded and integrated on PC installed with LC solutions chromatographic software. The Chromatographic separation was performed using HIBAR- 5µ [C18] column (250×4.6mm) as a stationary phase.

PREPARATION STANDARD STOCK SOLUTIONS

Stock solution was prepared by transferring10mg of Rivaroxaban in 10ml standard flask and it was dissolved in DMSO and made up to the mark. Further dilution was done by serial dilution method for obtaining respective concentration with acetonitrile.

SAMPLE PREPARATION

A total number of 20 tablets were weighed and the average weight was calculated. From the powdered tablets quantity equivalent to 10 mg was taken and it was dissolved in acetonitrile, sonicated, make up the volume to 10ml and filtered through whatmann filter paper, resulting solution was diluted to required concentration with appropriate dilutions.

METHOD DEVELOPMENT

Different ratios of mobile phases were tried for the separation and resolution. The various method optimization procedures were carried out and compared with system suitability parameters. The choice of wavelength 248nm was considered satisfactory, permitting the detection of the drugs with adequate sensitivity.

FIXED CHROMATOGRAPHIC CONDITIONS

After satisfactory optimizing procedure, 0.1M potassium dihydrogen orthophosphate (pH adjusted to 3.0 with orthophosphoric acid) and acetonitrile in the ratio of 60:40 % v/v used as the mobile phase. The flow rate was1.0 ml/min and the injector volume of the standard and sample was 20µl. The detection was carried out in the UV region at 248nm.

RESULTS AND DISCUSSION

METHOD VALIDATION

SYSTEM SUITABILITY

The developed HPLC method was analyzed for the system suitability parameters by injecting standard solutions. Parameters such as tailing factor, number of theoretical plates (N) and retention time (RT) were determined. The results of system suitability indicate better performance of system Table (1) (Fig.1).

LINEARITY

The linearity range of Rivaroxaban is 1-5µg/ml as showed in Table (2), (Fig.2).Under the experimental conditions described above, linear calibration curve obtained throughout the concentration ranges studied. Regression analysis was done on the peak area of the drug (y) v/s concentration (x).

ACCURACY

Accuracy of the method was determined by applying the proposed method to synthetic mixture containing known amount of drug to 50%, 100%, and 150% of the label claim. The accuracy was then calculated as the percentage of anasslyte recovered by the assay. The results of the recovery analysis are enclosed under Table (3).

PRECISION

The assay was carried out using proposed method in six replicates. The value of relative standard deviation lie well within the limits, it indicates the sample repeatability of the method enclosed in Table (4).

ROBUSTNESS

The robustness of the method was determined to check the reliability of an analysis with respect to deliberate variations in method parameters. The typical variations are given below: Variation in flow rate by ± 0.1ml/min, variation in mobile phase ratio by ± 0.1% and variation in pH of buffer ± 0.05.

DETECTION LIMIT AND QUANTITATION LIMIT (LOD AND LOQ)

The Detection Limit and Quantitation Limit can be calculated based on the Standard deviation of the response and the Slope. The results obtained are presented in Table (5).

METHOD APPLICATION

The validated high performance liquid chromatography method was applied for determination Rivaroxaban. Market available tablet dosage form contains Rivaroxaban 10mg. 20 tablets were weighed and the average weight was calculated. From the powdered tablets quantity equivalent to 10mg was taken and it was

dissolved in Acetonitrile. It was sonicated and volume made upto 10ml with acetonitrile and it was filtered through Whatmann filter paper. This solution was further diluted to get a solution having concentration of 3µg/ml Rivaroxaban. 20µl of this solution was injected into the HPLC system under the specified chromatographic conditions. The analyte peaks were identified by comparisons with those of respective standard for their retention time. The peak areas were used to calculate the concentration. The assay results expressed as % of the label claim in Table (6).

Table 1: System Performance for Rivaroxaban

Drug substance	Retention time	Tailing factor	No of Theoretical plates
Rivaroxaban	7.45 min	0.934	3138

Table 2: Linearity data for Rivaroxaban

S. No	Concentration (µg/ml)	Peak area (n=6)
1	1	38580
2	2	75938
3	3	101892
4	4	142182
5	5	169528
Correlation coefficient (r^2)		0.9978
Slope		32667.6
Intercept		7474.8

Table 3: Accuracy-%Recovery of Rivaroxaban

Sample ID	Concentration (µg/ml)		%Recovery of Pure drug	Statistical Analysis
	Pure drug	Formulation		
S1:50%	1.5	3	99.67	Mean= 99.22%
S2:50%	1.5	3	98.78	S.D. = 0.4450
S3:50%	1.5	3	99.23	% R.S.D.= 0.45
S4:100%	3	3	99.87	Mean= 99.54%
S5:100%	3	3	99.43	S.D. = 0.2910
S6:100%	3	3	99.32	% R.S.D.= 0.29
S7:150%	4.5	3	99.56	Mean= 99.15%
S8:150%	4.5	3	98.21	S.D. = 0.4206
S9:150%	4.5	3	99.18	% R.S.D.= 0.42

Table 4: Inter-day & Intra-day Precision of Rivaroxaban

S.No	Sample ID Concentration 3 µg/ml	Inter-day	Intra-day
1.	S1	101892	104532
2.	S2	103876	103769
3.	S3	102654	103876
4.	S4	103814	105642
5.	S5	101780	103943

6.	S6	102657	103456
		Mean= 102778.83	Mean= 104203
		S.D. = 904.4238	S.D. = 787.3824
		% R.S.D.= 0.88	% R.S.D.= 0.76

Table 5: LOD and LOQ of Rivaroxaban

1.	Limit of Detection concentration in µg/ml	0.0913
2.	Limit of Quantitation concentration in µg/ml	0.2768

Table 6: Assay of Rivaroxaban Tablets

Brand name of tablets	Labelled amount of Drug (mg)	Mean (±SD) Assay (n = 6)	% Label Claim (±SD)
Xarelto®	10	10.23 (±0.045)	102.30 (±0.12)

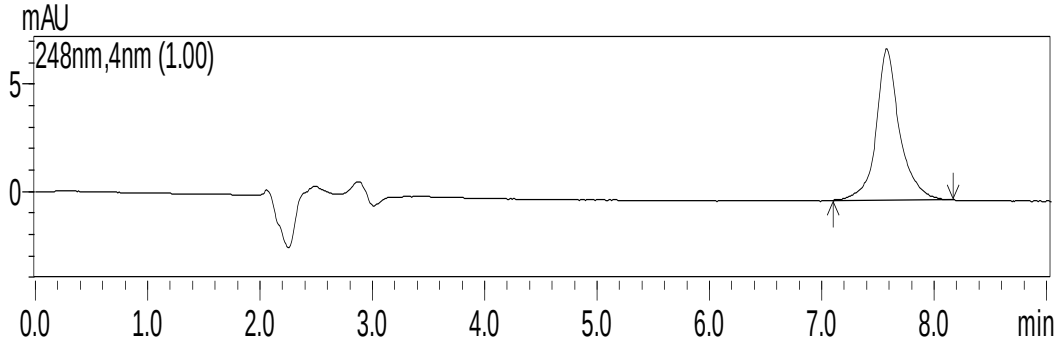

Fig. 1: Chromatogram of Rivaroxaban

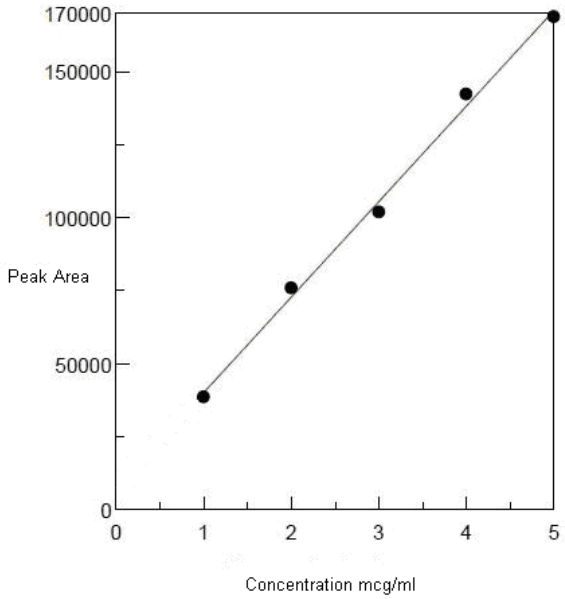

Fig. 2: Calibration graph of Rivaroxaban

CONCLUSION

In this study a simple, fast and reliable HPLC method was developed and validated for the determination of Rivaroxaban in tablet formulation. The proposed method has the lowest LOD values and is more sensitive method. From the results obtained, we concluded that the suggested methods showed high sensitivity, accuracy, reproducibility and specificity. This method is simple and inexpensive and can be employed for the routine quality control of Rivaroxaban in tablet formulation.

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