A NEW METHOD DEVELOPMENT AND VALIDATION FOR ANALYSIS OF RIVAROXABAN IN FORMULATION BY RP HPLC

K.Chandra sekhar, P. Satya vani , A. Dhana lakshmi, Ch.LL.Devi, Anupama Barik , Narendra Devanaboyina*

Department of Pharmaceutical Analysis, Lydia College of pharmacy, Ravulapalem533238, India. Email: narendra.kothapalli@gmail.com

ABSTRACT

A new simple, rapid, selective, precise and accurate isocratic reverse phase high performance liquid chromatography assay has been developed and validated for the estimation of Rivaroxaban in tablet formulation. The separation was achieved by using C-18 column (250x4.6mm, 5µm in particle size) at ambient temperature coupled with a guard column of silica in mobile phase Acetonitrile: Methanol: 0.1%Otho phosphoric acid (90:8:2) with the pH value adjusted to 4.06. The flow rate was 1.5ml/min and the drug was detected by using UV detector at the wavelength 234nm and the run time was 7min. The retention time was found 3.32 minutes. The percentage of RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per ICH guidelines. The proposed method was found to be accurate, repeatability and consistent. It can be successfully applied for the analysis of the drug in marketed formulation and could be effectively used for the routine analysis of the same drug without any alteration in the chromatographic conditions.

Keywords: Rivaroxaban, RP HPLC, UV Detection, 234nm, Validation, C18 column.

INTRODUCTION TO RIVAROXABAN: -

Rivaroxaban is a new antithrombotic drug that has been shown to provide superior efficacy to enoxaparin without increasing the risk of bleeding after major lower limb surgery. The introduction of this drug in a surgical department represents a paradigm change, since Rivaroxaban is not administered preoperatively but postoperatively, and is not given subcutaneously but orally.

Rivaroxaban can help prevent dangerous blood clots from forming after a hip or knee replacement surgery. It also prevents blood clots and strokes in people with an irregular heart rhythm known as atrial fibrillation. It comes in the form of a tablet that is taken 10mg once daily.

Some side effects of Rivaroxaban are potentially and serious reaction are related to bleeding an inherent risk with "blood-thinning"

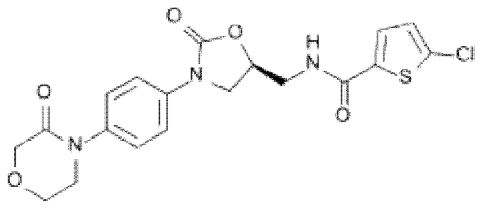


Figure 1: Structure of Rivaroxaban

IUPAC Name: *S*)-5-chloro-*N*-{[2-oxo-3-[4-(3-oxomorpholin-4-yl) phenyl] oxazolidin-5yl] methyl} thiophene-2-carboxamide.

EXPERIMENTAL

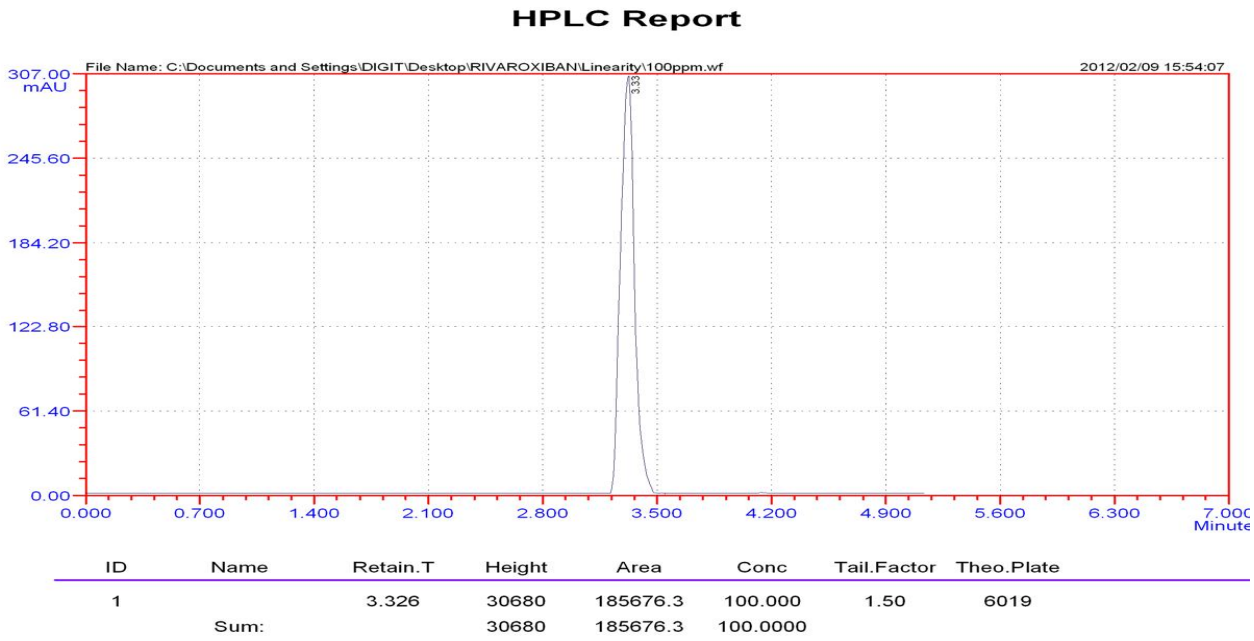
Working standard of **Rivaroxaban** was obtained from well reputed research laboratories. HPLC grade Acetonitrile: Methanol: 0.1%Ortho phosphoric acid (E.Merck, Mumbai, India).

TABLE-1

S.NO	PARAMETER	RESULT
1	Standard concentration	100µg/ml
2	Mobile phase	Acetonitrile :Methanol:Ortho phosphoric acid (90:8:2)
3	Wave length	234nm
4	pH	4.06
5	Flow rate	1.5 ml/min.
6	Retention time	3.326min.
7	Run time	7min.
8	Peak area	185676.3
9	Theoretical plates	6018.81
10	Pump pressure	4.9psi

Chromatographic conditions of Rivaroxaban

Figure: 1



Sample chromatogram of Rivaroxaban in HPLC

Different mobile phases were tried and the separation, well resolved and good symmetrical peaks were obtained with the mobile phase Acetonitrile: Methanol: 0.1%Ortho phosphoric acid (90:8:2). The retention time of rivaroxaban was found to be 3.33min with pressure 4.9 psi, which shows satisfactory result. The system suitability and validation parameters are given in Table 1. The high percentage of recovery of rivaroxaban was found to be 100.57% indicating that the proposed method is highly accurate and precise. Present liquid chromatographic method was applied for the determination of rivaroxaban in tablet formulation. The result for rivaroxaban was comparable with a corresponding labeled amount (Table 7). The absence of additional peaks indicates no interference of the excipients.

Chemicals and Reagents

The Tablets of combined dosage form were procured from the local market. Other reagents used like Acetonitrile, Methanol, 0.1%Ortho phosphoric acid ,which are of HPLC grade were purchased from E.Merck, Mumbai, India

Analytical conditions

The development and validation of the assay was performed on a Series HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column zodiac C18 (250×4.6mm), and manual injector Rheodyne valve with (20µL) fixed loop, PEAK LC software was used.

The mobile phase consists of a Acetonitrile: Methanol :0.1%Ortho phosphoric acid (90:8:2) and injections were carried out by using 20µl loop at room temperature, flow rate 1.5ml/min. absorbance at 234 nm with runtime 7min.

Instrumental Apparatus

A Series HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column zodiac C18(250×4.6mm), Electronic balance-DENVER (SI234),manual Rheodyne injector(20µl)loop PEAK LC software were used. UV 2301 SPECTROPHOTOMETER was used to determine the wavelength of maximum absorbance.

Determination of wavelength of maximum absorbance

The standard solutions of rivaroxaban were scanned in the range of 200-400nm against mobile phase as a blank. Rivaroxaban showed maximum absorbance at 234nm. So the wavelength selected for the determination of rivaroxaban was 234nm.

Preparation of Stock, working standard solutions

10 mg of Rivaroxaban reference substance was accurately weighed and dissolved in 10 ml of mobile phase in a 100 ml volumetric flask to obtain 1000ppm solution. From standard solution by serial dilution we prepared required concentration of 200 ppm.

Preparation of Sample solutions

A composite of 20 tablets was prepared by grinding them to a fine, uniform size powder. 10 mg of Rivaroxaban was accurately weighted and quantitatively transferred into a 100ml volumetric flask. Approximately 50ml mobile phase were added and the solution was sonicated for 15min. The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 100µg/ml.

Method validation procedure

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines for linearity, precision, system suitability, specificity, limit of detection and limit of quantification and robustness.

Range of Linearity

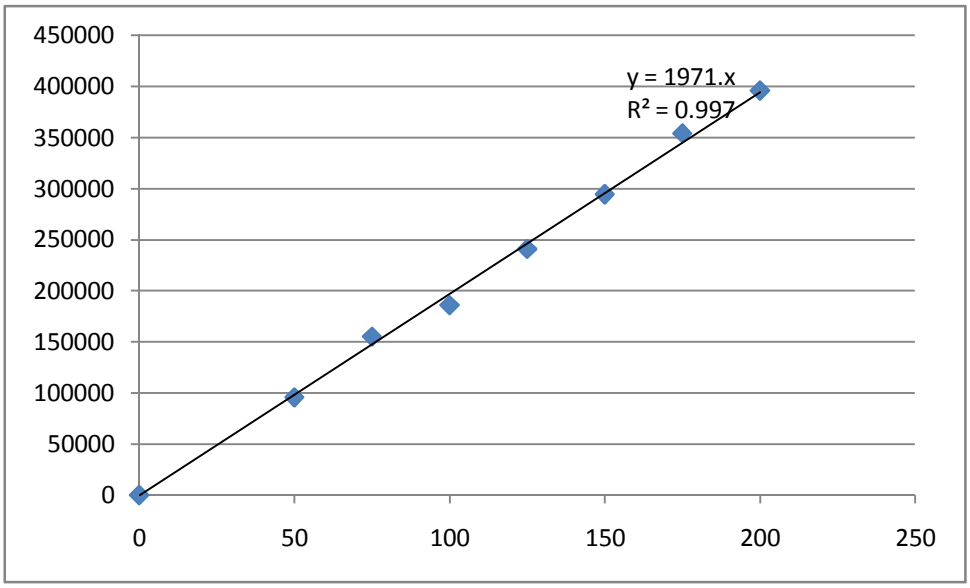
Standard curves were constructed daily, for three consecutive days, using nine standard concentrations in a range of 50, 75, 125, 150, 175, 200 µg/ml for rivaroxaban. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y = -2634+ 1971x (r= 0.997). Linearity values can shown in Table: 2

TABLE:-2 **LINEARITY**

S.NO	CONCENTRATION	AREA
1	50ppm	95444.8
2	75ppm	154956.3
3	100ppm	185676.3
4	125ppm	240639.5
5	150ppm	294114.6
6	175ppm	353703.0
7	200ppm	395479.3

Graph:-1

LINEARITY GRAPH



Calibration curve of Rivaroxaban

Precision

To study precision, six replicate standard solutions of rivaroxaban (100ppm) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated and it was found to be which is well within the acceptance criteria of not more than 2.0%. Results of system precision studies are shown in table:

TABLE:-3

PRECISION TABLE

S.NO	CONCENTRATION	AREA	%RSD
1	100ppm	186388.7	0.471%
2	100ppm	184117.6	
3	100ppm	185300.5	
4	100ppm	184990.5	
5	100ppm	185043.5	
6	100ppm	186346.8	

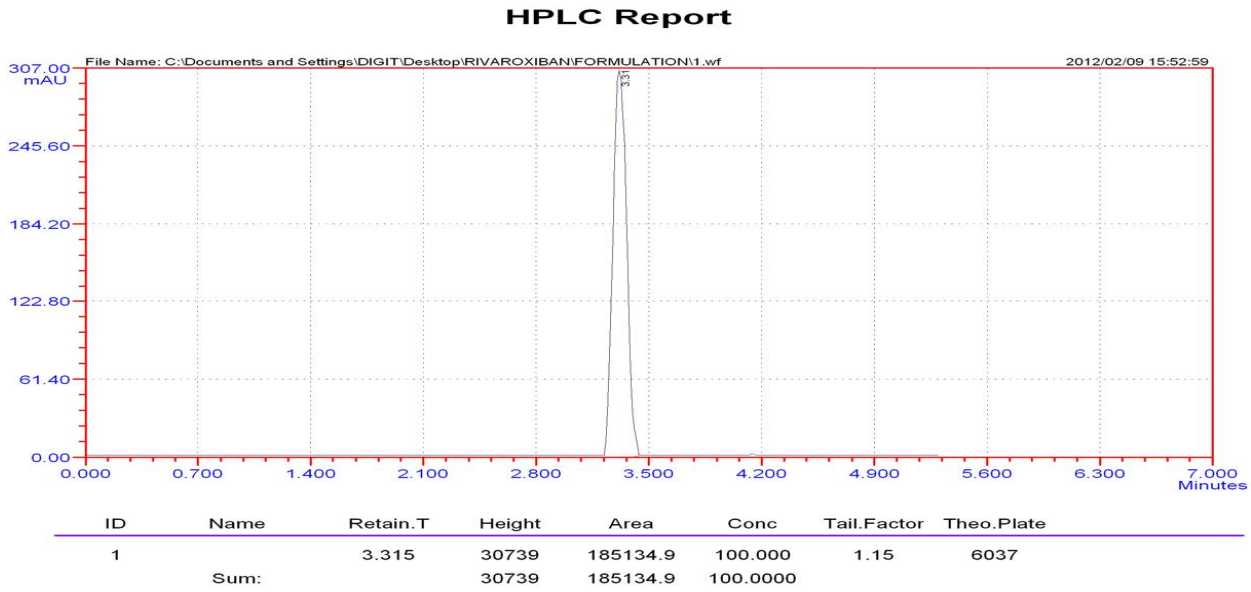
RUGGEDNESS

S.NO	CONCENTRATION	AREA	%RSD
1	100ppm	184080.1	0.609%
2	100ppm	184614.3	
3	100ppm	184463.0	
4	100ppm	183561.0	
5	100ppm	185238.9	
6	100ppm	186789.4	

System Suitability

Having optimized the efficiency of a chromatographic separation the quality of the chromatography was monitored by applying the following system suitability tests: capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2000. In all cases, the relative standard deviation (R.S.D) for the analytical peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution is presented. System suitability parameters were shown in Table.1. Standard chromatogram was given in Figure.2

Figure:2



Standard chromatogram of Rivaroxaban

Specificity

The specificity of the method was determined by comparing test results obtained from analysis of sample solution containing excipients with that of standard results those obtained from standard drug.

Limit of Detection and Limit of Quantification:

To determine the Limit of Detection (LOD) sample was dissolved by using Mobile phase and injected until peak was disappeared. After 5ppm dilution Peak was not clearly observed, based on which 5ppm is considered as Limit of Detection and Limit of Quantification is 2.47ppm.

Limit of detection (LOD):

The Limit of Detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value.LOD can be calculated as:

LOD = 0.75ppm

TABLE: 4

Limit of detection and limit of quantification;

1	LOD	0.75ppm
2	LOQ	2.47ppm

Limit of quantification (LOQ):

The Limit of Quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined with suitable precision and accuracy. (LOQ = LOD × 3.3)

LOQ = 2.47ppm

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. In this study, the chromatographic parameters monitored were retention time, tailing factor and theoretical plates. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above and the results are given in Table 4

Table 5:

PARAMETER CHANGED	**CHANGE**	**AREA**	**AMOUNT RECOVERED**	%OF CHANGE
STANDARD	.....	185676.3	.....	.....
MOBILE PHASE	Acetonitrile:Methanol: 0.1%Orthophosphoricacid (85: 13:2)	185791.8	100.06	0.06
WAVE LENGTH	240	186343.0	100.35	0.35
FLOW RATE	2	184231.5	99.22	0.78

Robustness parameter of RIVAROXABAN

Recovery

Recovery test was performed at 3 different concentrations i.e. 50ppm, 100ppm, 150ppm. Results are given in table-6.

Table: 6

S. NO.	CONCENTRATION	AMOUNT FOUND	% RECOVERY
1	50ppm	50.489	50.489
2	100ppm	100.44	100.44
3	150ppm	151.79	151.79
Average recovery			100.573

RESULT AND DISCUSSION

Optimization of the chromatographic conditions

The Sample nature, its solubility and molecular weight confirms the correct selection of the stationary phase. The drug rivaroxaban being non-polar is preferably analyzed by reverse phase columns and accordingly C18 column was selected. The elution of the compound from the column was influenced by polar mobile phase. The concentration of the Acetonitrile: Methanol: 0.1%Ortho phosphoric acid (90:8:2), were optimized to give symmetric peak with short run time based on asymmetric factor and peak area obtained.

ASSAY

TABLET FORMULATION

Formulation	Dosage	Concentration	Amount found	% ASSAY
Xraelto(Tablet)	10mg	100 ppm	99.708 ppm	99.708

CONCLUSION

The proposed method for the assay of rivaroxaban in tablets or capsules is very simple and rapid. It should be emphasized that, it is isocratic and the mobile phase do not contain any buffer. This method was validated for linearity, precision, system suitability, specificity, LOD & LOQ and robustness. Although the method could effectively separate the drug from its products, further studies should be performed in order to use it to evaluate the stability of pharmaceutical formulations.

ACKNOWLEDGEMENT

With profound sense of gratitude and humbleness we express our sincere thanks to our dedicated Supervisor, Faculty members and Management of Lydia College of pharmacy, Ravulapalem, E.G Dist., Andhrapradesh, India.

REFERENSES

1. Roehrig S, Straub A, Pohlmann J, *et al.* (September 2005). "Discovery of the novel antithrombotic agent (BAY 59-7939): an oral, direct factor Xa inhibitor". *Journal of Medicinal Chemistry* **48** (19): 5900–8.
2. Eriksson BI, Borris LC, Dahl OE, *et al.* (November 2006). "A once-daily, oral, direct Factor Xa inhibitor, rivaroxaban (BAY 59-7939), for thromboprophylaxis after total hip replacement". *Circulation* **114** (22): 2374–81.
3. Eriksson BI, Borris LC, Friedman RJ, *et al.* (June 2008). "Rivaroxaban versus enoxaparin for thromboprophylaxis after hip arthroplasty". *The New England Journal of Medicine* **358** (26): 2765–75.
4. Kakkar AK, Brenner B, Dahl OE, *et al.* (July 2008). "Extended duration rivaroxaban versus short-term enoxaparin for the prevention of venous thromboembolism after total hip arthroplasty: a double-blind, randomised controlled trial". *Lancet* **372** (9632): 31–9.
5. Lassen MR, Ageno W, Borris LC, *et al.* (June 2008). "Rivaroxaban versus enoxaparin for thromboprophylaxis after total knee arthroplasty". *The New England Journal of Medicine* **358** (26): 2776–86. doi:10.1056/NEJMoa076016. PMID 18579812.
6. Turpie A, Bauer K, Davidson B, *et al.* (2008). "Comparison of rivaroxaban – an oral, direct factor Xa inhibitor – and subcutaneous enoxaparin for thromboprophylaxis after total knee replacement (RECORD4: a phase 3 study)". *European Federation of National Associations of Orthopaedics and Traumatology Annual Meeting; May 29 – June 1, 2008; Nice, France, Abstract F85*. http://proceedings.jbjs.org.uk/cgi/content/abstract/92-B/SUPP_II/329-a.
7. Turpie AG, Lassen MR, Davidson BL, *et al.* (May 2009). "Rivaroxaban versus enoxaparin for thromboprophylaxis after total knee arthroplasty (RECORD4): a randomised trial". *Lancet* **373** (9676): 1673–80. doi:10.1016/S0140-6736(09)60734-0.

8. ClinicalTrials.gov. "Randomized, Double-Blind Study Comparing Once Daily Oral Rivaroxaban With Adjusted-Dose Oral Warfarin for the Prevention of Stroke in Subjects With Non-Valvular Atrial Fibrillation". http://clinicaltrials.gov/ct2/results?term=NCT00403767. Retrieved 2009-02-11.
9. ClinicalTrials.gov. "MAGELLAN - Multicenter, Randomized, Parallel Group Efficacy Superiority Study in Hospitalized Medically Ill Patients Comparing Rivaroxaban with Enoxaparin". http://clinicaltrials.gov/ct2/show/NCT00571649?term=rivaroxaban&rank=11. Retrieved 2009-02-11.
10. ClinicalTrials.gov. "Once-Daily Oral Direct Factor Xa Inhibitor Rivaroxaban in the Long-Term Prevention of Recurrent Symptomatic Venous Thromboembolism in Patients With Symptomatic Deep-Vein Thrombosis or Pulmonary Embolism. The Einstein-Extension Study". http://clinicaltrials.gov/ct2/show/NCT00439725?term=rivaroxaban&rank=5. Retrieved 2009-02-11.
11. ClinicalTrials.gov. "Oral Direct Factor Xa Inhibitor Rivaroxaban In Patients With Acute Symptomatic Deep-Vein Thrombosis (DVT) Without Symptomatic Pulmonary Embolism: Einstein-DVT Evaluation". http://clinicaltrials.gov/ct2/show/NCT00440193?term=einstein+rivaroxaban&rank=2. Retrieved 2009-02-11.