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A NOVEL RP-HPLC METHOD FOR THE QUANTIFICATION OF RIVAROXABAN IN FORMULATIONS

DR. NALLAGATLA. VIJAYA BHASKAR RAO* AND BRC SEKHAR REDDY

Principal, Govt.Degree College, Amrabad, Mahabubnagar.Dist, A.P, India, Pin: 509201.
Micro Labs Limited-API, # 43-45 KIADB Industrial Area Phase-IV, Bommasandra, Bangalore- 560 105.

ABSTRACT

A simple, precise and accurate RP-HPLC method was developed and validated for assay of Rivaroxaban in tablet dosage form. Isocratic elution at a flow rate of 1ml/min was employed on a Kromasil C18 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted of Methanol: Acetonitrile 80: 20 v/v, (P^H 4.4). The UV detection wavelength was 273 nm and 20µl sample was injected. The retention time for Rivaroxaban was 5.35 min. The percentage RSD for precision and accuracy of the developed method was found to be less than 2%. The newly developed method was validated as per the ICH guidelines. The method was successfully applied for regular analysis of Rivaroxabanin in different formulations and bulk drug.

KEY WORDS: **Rivaroxaban, RP-HPLC, UV detection, recovery, precise, 273nm.**

DR. NALLAGATLA. VIJAYA BHASKAR RAO

Principal, Govt.Degree College, Amrabad, Mahabubnagar.Dist, A.P, India, Pin: 509201.

INTRODUCTION

Rivaroxaban is an oral anticoagulant invented and manufactured by Bayer.[1] This medicine is used for several days after hip or knee replacement surgery while you are unable to walk. It is during this time that blood clots are most likely to form. On July 1, 2011, the U.S. Food and Drug Administration (FDA) approved Rivaroxaban for prophylaxis of deep vein thrombosis (DVT), which may lead to pulmonary embolism (PE), in adults undergoing hip and knee replacement surgery.(1, 2, 3, 4) Rivaroxaban is used to prevent deep venous thrombosis, a condition in which harmful blood clots form in the blood vessels of the legs. These blood clots can travel to the lungs and can become lodged in the blood vessels of the lungs, causing a condition called pulmonary embolism. It is also used to prevent stroke and blood clots in patients with certain heart rhythm problem (e.g., nonvalvular atrial fibrillation).

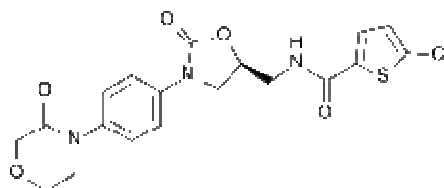


Figure 1
Structure of Rivaroxaban

Systematic (IUPAC) name: (S)-5-chloro-N-{[2-oxo-3-[4-(3-oxomorpholin-4-yl) phenyl]oxazolidin-5-yl]methyl}thiophene-2-carboxamide
Formula : $C_{19}H_{18}ClN_3O_5S$
Mol. mass : 435.882 g/mol
Routes : Oral

Rivaroxaban is an oxazolidinone derivative optimized for inhibiting both free Factor Xa and Factor Xa bound in the prothrombinase complex.[5] It is a highly selective direct Factor Xa inhibitor with oral bioavailability and rapid onset of action. Inhibition of Factor Xa interrupts the intrinsic and extrinsic pathway of the blood coagulation cascade, inhibiting both thrombin formation and development of thrombi. Rivaroxaban does not inhibit thrombin (activated Factor II), and no effects on platelets have been demonstrated.[6] Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in clinical practice.(7-20) The most common adverse reactions with Rivaroxaban were bleeding complications. The following adverse reactions have been identified during post-approval use of rivaroxaban. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure. Those are Blood and lymphatic system disorders- agranulocytosis, Gastrointestinal disorders: retroperitoneal hemorrhage, Hepatobiliary disorders- jaundice, cholestasis, cytolytic hepatitis, Immune system disorders- hypersensitivity, anaphylactic reaction, anaphylactic shock, Nervous system disorders- cerebral hemorrhage, subdural hematoma, epidural hematoma, hemiparesis, Skin and subcutaneous tissue disorders- Stevens-Johnson syndrome.

EXPERIMENTAL

Materials

Working standard of Rivaroxaban was obtained from well reputed research

laboratories. HPLC grade Acetonitrile, Methanol was purchased from E. Merck (Mumbai, India).

Apparatus

A Series HPLC [6-11] system PEAK LC 7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Kromasil C18. 250×4.6mm, Electronic balance-DENVER (SI234), manual Rheodyne injector with a 20 µl loop was used for the injection of sample. PEAK LC software was used. UV 2301 Spectrophotometer was used to determine the wavelength of maximum absorbance.(21,22)

Determination of wavelength of maximum absorbance

The standard solutions of Rivaroxaban were scanned in the range of 200 - 400 nm against mobile phase as a blank. Rivaroxaban showed maximum absorbance at 273nm. So the wavelength selected for the determination of Rivaroxaban was 273nm.

Chromatographic equipment and conditions

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of RIVAROXABAN an isocratic PEAK HPLC instrument with Kromasil C18 column (250 mm x 4.6 mm, 5µ) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC – 7000 UV-detector. A 20µL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software. The mobile phase consisted of Methanol: Acetonitrile 80: 20 (v/v), (P^H 4.4) Injections were carried out using a 20 µl loop at room temperature (20 + 2 °C) and the flow rate was 1 ml/min. Detection was performed at 273nm with 10min run time.

Standard and sample solutions

A 10 mg amount of Rivaroxaban reference substance was accurately weighed and dissolved in 10 ml mobile phase in a 10 ml volumetric flask to obtain 1000 ppm concentrated solution. Required concentrations were prepared by serial dilution of this solution. A composite of 20 (XARELTO-10mg) tablets was prepared by grinding them to a fine, uniform size powder. 10 mg of Rivaroxaban was accurately weighed and quantitatively transferred into a 100 ml volumetric flask. Approximately 25 ml mobile phase were added and the solution was sonicated for 15 min. 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 60ppm.

Method validation

Method validation was performed following ICH specifications for system suitability, specificity, range of linearity, LOD, LOQ, accuracy, precision and robustness.

RESULTS AND DISCUSSION

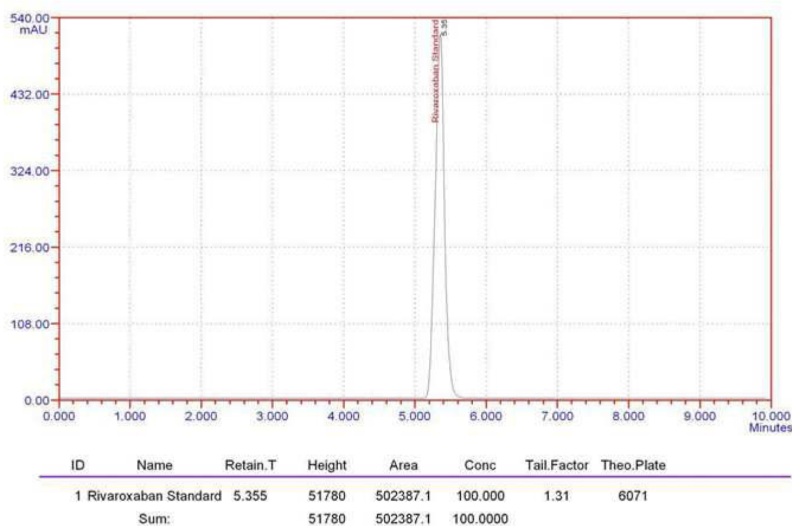
System Suitability

Having optimized the efficiency of a chromatographic separation, the quality of the chromatograph 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 > 2500. In all cases, the % relative standard deviation (R.S.D) for the analytic 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.

Table 1
System suitability parameters of Rivaroxaban.

Api Conc.	60 ppm
Mobile Phase	Methanol: Acetonitrile 80: 20 (v/v)
Wavelength	273nm
Column	C_{18} Column
P^H	4.4
Retention Time	5.35min
Run Time	10min
Area	502387
Theoretical Plates	6071
Tailing Factor	1.31
Pump Pressure	7.6 MPa

HPLC Report



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	Rivaroxaban Standard	5.355	51780	502387.1	100.000	1.31	6071
	Sum:		51780	502387.1	100.0000		

Figure 2
Standard chromatogram of Rivaroxaban.

Range of linearity

Standard curves were constructed daily, for three consecutive days, using ten standard concentrations in a range of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100ppm for Rivaroxaban. The linearity of peak area sample versus sample concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y = 18504 + 8100x (r= 0.999). Linearity values can show in Table.2.

Table 2
Linearity results of Rivaroxaban.

S. No.	Conc. (ppm)	Area
1	10	99891
2	20	186828
3	30	274407
4	40	347220
5	50	414829
6	60	502387
7	70	599999
8	80	679175
9	90	743721
10	100	810365
	Slope	8100.488
	Intercept	18504.86
	CC	0.999056

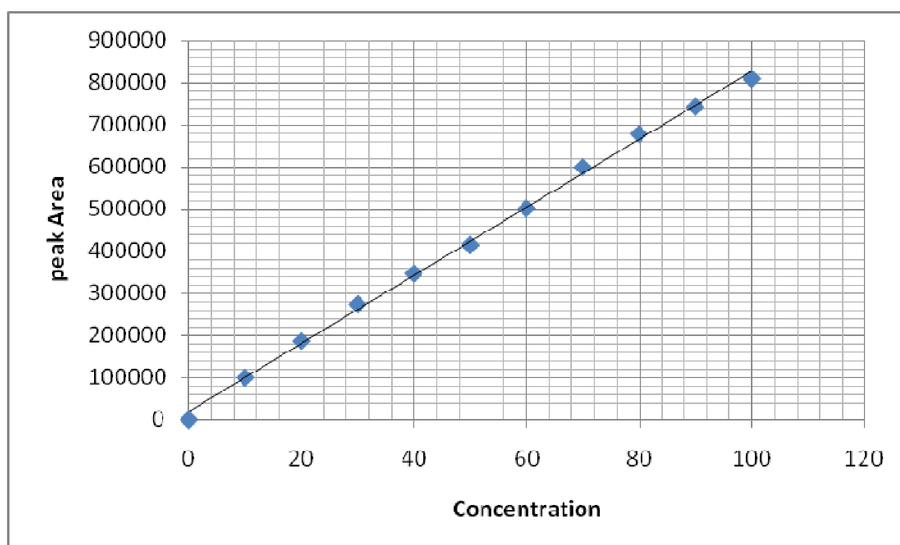


Figure 3
Calibration curve of Rivaroxaban.

Precision
To study precision, six replicate sample solutions of Rivaroxaban (60ppm) 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.3 and Table.4.

Table 3
Intraday Precision Results for Rivaroxaban.

Sample preparation No. (API 60 µg/ml)	Area
1	502429
2	503531
3	502237
4	503586
5	501602
6	503274
RSD	**0.16**

Table 4
Inter day Precision results of Rivaroxaban.

Sample preparation No. (API 60 µg/ml)	Area
1	501259
2	503023
3	499405
4	500435
5	501229
6	500177
RSD	**0.24**

Limit of Detection and Limit of Quantification
To determine the Limit of Detection sample was dissolved by using Mobile phase and injected until peak was disappeared. After 0.5ppm dilution Peak was not clearly observed, based on which 0.5ppm is considered as Limit of Detection and Limit of Quantification is 1.5ppm.

Table 5
LOD and LOQ results of Rivaroxaban.

Parameter	Measured Value
Limit of Quantification	1.5ppm
Limit of Detection	0.5ppm

Robustness

Typical variations in HPLC conditions were used to evaluate the robustness of the developed method. The robustness study was performed by slight modification in flow rate of the mobile phase, composition of the mobile phase and wavelength of the detector. Rivaroxaban at standard concentration was analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above. Results were shown in Table.6.

Table 6
Robustness results of Rivaroxaban.

S. No.	Parameter	Change	Area	% of Change
1	Standard	No change in mobile phase preparation.	502387	…………
2	MP	Methanol: Acetonitrile		
		Mp-1 85:15	499023	0.66
		Mp-2 75:25	501255	0.22
3	pH	4.3	503877	0.29
		4.5	506513	0.82
4	WL	278nm	497669	0.93
		268nm	500870	0.30

Ruggedness

Ruggedness was performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different. Ruggedness also expressed in terms of percentage relative standard deviation.

Sample (µg/ml)	Area
1	505858
2	506899
3	503484
4	503596
5	504043
6	504579
RSD	**0.27**

Recovery

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. Recovery test was performed at 3 different concentrations i.e. 30ppm, 40ppm, 50ppm. The percent recovery was calculated and results are presented in Table. Satisfactory recoveries ranging from 98.9 to 101.6 were obtained by the proposed method. This indicates that the proposed method was accurate. Results are given in Table.8

Table 8
Recovery results of Rivaroxaban.

% Recovery	Rivaroxaban				
	Target Conc. (ppm)	Spiked conc. (ppm)	Final Conc. (ppm)	Conc. Obtained	% of Recovery
50%	20	10	30	30.4	101.6
	20	10	30	30.3	101.3
	20	10	30	30.2	100.6
100%	20	20	40	39.5	98.9
	20	20	40	40.3	100.7
	20	20	40	40.2	100.6
150%	20	30	50	49.9	99.8
	20	30	50	50.03	100.01
	20	30	50	49.5	99.1

Degradation studies

Forced degradation studies of both the drugs were carried out under conditions acid, alkali, peroxide, heat, Sun light, uv light, aqueous etc. After exposing sample was tested immediately and 48 hours incubation. It can be concluded that the method separates the drugs from their degradation products. It may be employed for analysis of stability samples of Rivaroxaban. Degradation studies are given in Table.9.

Table 9
Degradation studies.

Condition after 48 hours	Observation
Standard	No degradation
3% Peroxide	Rivaroxaban degraded in to four compounds
0.1 N Basic	Rivaroxaban degraded in to three compounds
0.1 N Acidic	Rivaroxaban degraded in to two compounds
Sun light	Rivaroxaban degraded in to three compounds
UV light	Rivaroxaban degraded in to three compounds
Aqueous (HPLC)	Rivaroxaban degraded in to one compound
Thermal (thermal)	Standard peak was split into four peaks

Stability studies

Stability test was conducted by injecting the sample solution in different time intervals after preparation. The sample showed the stable up to 48 hours after preparation. Stability studies are given in Table.10.

Table 10
Stability studies.

S. No.	Time In hours	Area Obtained	% Assay
1	0	501949	99.91
2	1	499988	99.52
3	2	496917	98.91
4	4	495709	98.67
5	6	500641	99.65
6	12	498085	99.14
7	18	494869	98.50
8	24	495432	98.62
9	36	493019	98.13
10	**48**	**489378**	**97.41**

Table 11
Formulation analysis

Formulation	Brand name & Dosage	Sample conc.	Area	% Assay	Amount found
Rivaroxaban	Xarelto-10mg	60ppm	496156	98.76	59.2

CONCLUSION

The proposed method for the assay of Rivaroxaban in tablets or capsules is very simple and rapid. It should be emphasized it is isocratic and the mobile phase do not contain any buffer. The method was validated for specificity, linearity, precision, accuracy and robustness. The proposed method gave good resolution of Rivaroxaban and its degradants. There was no significant change in analyte composition over a period of 36 h. System suitability tests and statistical analysis performed prove that the method is precise, accurate and reproducible, and hence can be employed for routine analysis of Rivaroxaban in bulk and commercial formulations.

REFERENCES

1. "FDA Approves Xarelto® (Rivaroxaban tablets) to Help Prevent Deep Vein Thrombosis in Patients Undergoing Knee or Hip Replacement Surgery" (Press release). Janssen Pharmaceutica. 2011-07-01. Retrieved 2011-07-01.
2. "Bayer's Xarelto Approved in Canada" (Press release). Bayer. 2008-09-16. Retrieved 2010-01-31.
3. "Bayer's Novel Anticoagulant Xarelto now also approved in the EU" (Press release). Bayer. 2008-02-10. Retrieved 2010-01-31.
4. Bayer. Bayer's Xarelto approved in the EU for the prevention of stroke in patients with AF and in the treatment of DVT [press release]. December 19, 2011
5. Roehrig S, Straub A, Pohlmann J, et al. (September 2005). "Discovery of the novel antithrombotic agent 5-chloro-N-({(5S)-2-oxo-3- [4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl}methyl)thiophene- 2-carboxamide (BAY 59-7939): an oral, direct factor Xa inhibitor". Journal of Medicinal Chemistry 48 (19): 5900–8.
6. "Xarelto: Summary of Product Characteristics". Bayer Schering Pharma AG. 2008. Retrieved 2009-02-11.
7. 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.
8. 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.
9. 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.
10. 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 (Record 4: a phase 3 study)". European Federation of National Associations of Orthopaedics and Traumatology Annual Meeting; May 29 – June 1, 2008; Nice, France, Abstract F85 92-B (SUPP II): 329.
11. Turpie AG, Lassen MR, Davidson BL, et al. (May 2009). "Rivaroxaban versus enoxaparin for thromboprophylaxis after total knee arthroplasty (Record 4): a randomised trial". Lancet 373 (9676): 1673–80.
12. Dabigatran, rivaroxaban, or apixaban versus enoxaparin for thromboprophylaxis after total hip or knee replacement: systematic review, meta-analysis, and indirect treatment comparisons BMJ 2012; 344 Antonio Gómez-Outes et al doi: 10.1136/bmj.e3675
13. Hughes, Sue (5 April 2011). "MAGELLAN: Rivaroxaban prevents VTE in medical patients, but bleeding an

issue". theheart.org. Retrieved 15 April 2011.
14. "About the MAGELLAN Study". Bayer HealthCare. Retrieved 15 April 2011.
15. ClinicalTrials.gov. "MAGELLAN - Multicenter, Randomized, Parallel Group Efficacy Superiority Study in Hospitalized Medically Ill Patients Comparing Rivaroxaban with Enoxaparin". Retrieved 2009-02-11.
16. Bauersachs, M.D., Rupert; The EINSTEIN Investigators (December 23, 2010). "Oral Rivaroxaban for Symptomatic Venous Thromboembolism". The New England Journal of Medecine 363 (26): 2499–2510. Retrieved 4 April 2011.
17. "Oral Direct Factor Xa Inhibitor Rivaroxaban In Patients With Acute Symptomatic Deep-Vein Thrombosis Without Symptomatic Pulmonary Embolism: Einstein-DVT Evaluation". clinicaltrials.gov. Retrieved 15 April 2011.
18. ClinicalTrials.gov. "Oral Direct Factor Xa Inhibitor Rivaroxaban In Patients With Acute Symptomatic Pulmonary Embolism (PE) With Or Without Symptomatic Deep-Vein Thrombosis: Einstein-PE Evaluation". Retrieved 2009-02-11
19. Rivaroxaban and usual care had similar rates of recurrent VTE and bleeding in symptomatic PE Daniel M. Witt, PharmD, FCCP, BCPS.
20. 20.S. Adam et al Comparative effectiveness of warfarin and new oral anticoagulants for the management of atrial fibrillation and venous thromboembolism: a systematic review.
21. P.V.V. Satyanarayana, Alavala Siva Madhavi, LCMS METHOD devlopment and validation ofpaliperidone in formulation dosage, Research Desk, 2012, July-Sept 1(1).1-9
22. Jujjuri padma, Regunta hamalatha, Diyi.D.V.S.N.N Manikanteswari, Chinnikrishna Davudala, Ummadi satya vathi, and Rambabu.Kuchi, A Novel RP-HPLC method for the quantification of roflumilast in formulations, The Experiment, Feb.2013 Vol..7 (2),388-394