View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by CiteSeerX

http://www.e-journals.in
DOI:10.7598/cst2014.893

Chemical Science Transactions
2014, **3(4),** 1546-1554

RESEARCH ARTICLE

Application of Stability Indicating HPLC Method with UV Detector to the Analysis of Rivaroxaban in Bulk and Tablet Dosage Form

BURLA SUNITHA VENKATA SESHAMAMBA[1*], PERURI VEERA VENKATA SATYANARAYANA[2] and CHANDRA BALA SEKARAN[3]

[1]Department of Food Chemistry and Nutrition, College of Food Science and Technology, Bapatla, Andhra Pradesh-522101, India

[2]Department of Biochemistry, Acharya Nagarjuna University, Guntur, Andhra Pradesh-522510, India

[3]Department of Biotechnology, Jagarlamudi Kuppuswamy Choudary College, Guntur, Andhra Pradesh-520010, India

sunithafst@gmail.com

Received 28 May 2014 / Accepted 29 June 2014

Abstract: A simple and sensitive stability indicating HPLC method is developed for the quantification of rivaroxaban in bulk and tablet dosage form. Chromatographic separation was achieved on an ACE-Ciano column (250 mm x 4.6 mm, 5 µm particle size). The mobile phase consists of 0.1M sodium acetate and methanol (60:40 *v/v*) and was delivered at a flow rate of 1 mL/min. A UV detector was used for the detection. The rivaroxaban was subjected to stress conditions for the assessment of the stability-indicating nature of the method. The method was validated as per ICH guidelines. The linearity is obtained in the range of 1-120 µg/mL. The limit of detection and quantification values is 0.194 µg/mL and 0.648 µg/mL respectively. The intra and inter-day %RSD values were below 1%. Intra and inter-day accuracies were within 100.10% and 100.40%, respectively. Degradation products resulting from the stress studies have no interfere with the detection of rivaroxaban. The average recovery of rivaroxaban in tablet dosage form was 99.74% with %RSD of 0.421%. The developed method was proved adequate for quantitative determination of rivaroxiban in presence of its degradation products.

Keywords: Analysis, HPLC, Rivaroxaban, Stability indicating, Tablets

Introduction

Rivaroxaban (RXN), (S)-5-Chlor-*N*-{2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-lmethyl}thiophen-2-carbamid, is a novel, oral, direct Factor Xa inhibitor approved for the prevention of venous thromboembolism in adult patients who have undergone hip or knee replacement surgery[1,2].

Muralikrishna and Kasad[3], Sekaran *et al.*[4] Satyanarayana and Madhavi[5] have determined RXN in formulations using spectrophotometry. Satyanarayana and Madhavi[6] and Sekhar *et al.*[7] have reported RP-HPLC method for the determination of RXN in formulations. Assay of RXN in human plasma by HPLC-MS/MS has been reported by Rohde[8]. Anti-factor Xa chromogenic assay method have been applied for quantification of RXN in human plasma by Mani *et al.*[9] Asmis *et al.*[10] Samama *et al.*[11] and Harenberg *et al.*[12] Stability indicating HPLC method is the most preferential technique for the analysis of drugs in the presence of its degradants. Celebier *et al.*[13] and Kasad and Muralikrishna[14] described stability indicating HPLC methods for the quantification of RXN.

The present manuscript describes a sensitive, selective, precise and accurate stability indicating HPLC method for the quantification of RXN in tablet formulations in accordance with ICH guidelines[15,16].

Experimental

The chromatographic analysis was performed with Waters Alliance HPLC system equipped with 2695 separation modules having 2996 photodiode array detector. HPLC analysis was conducted in an ACE-Ciano column (250 mm x 4.6 mm, 5 µm particle size).

Chemicals

Solvents and chemicals used were of HPLC and analytical grade, respectively. Pharmaceutical grade RXN was kindly supplied by Rainbow Pharma Training Lab, Hyderabad. Xeralto tablets (Bayer India Limited Mumbai, India) claimed to contain 10 mg of RXN were purchased from the local pharmacy store.

Mobile phase preparation

The mobile phase is composed of 0.1M sodium acetate (Merck, Mumbai, India) and methanol (Thermo Fisher Scientific India Pvt. Ltd, Mumbai, India) in the ratio of 60:40 (*v/v*). The pH of the mobile phase was adjusted to 5.5 with 85% orthophosphoric acid (Thermo Fisher Scientific India Pvt. Ltd, Mumbai, India). The mobile phase was degassed and filtered via 0.45 μm membrane filter before use.

Stock Standard solution preparation

A stock standard solution of RXN (1 mg/mL) was prepared in the mobile phase.

Chromatographic conditions

The HPLC parameters such as run time 6 minutes, 1 mL/min as flow rate, injection volume of 10 µL and column temperature of 30 °C were finalized during development. RXN was detected at 247nm.

General assay procedure

The stock standard solution (1 mg/mL) was diluted with mobile phase to obtain working standard solutions in the concentration range of 1–120 µg/mL RXN. Each concentration was injected into the HPLC system thrice. The mean peak area versus corresponding concentrations of RXN was plotted and the calibration curve was drawn. The concentration of the RXN in unknown samples was read from the calibration curve or computed from the regression equation.

Analysis of RXN in tablets

Twenty tablets were weighed and subsequently powdered. An accurately weighed amount of powder equivalent to 50 mg RXN was transferred into a 50 mL volumetric flask and dissolved

in 20 mL of mobile phase. This mixture was sonicated for 15 minutes and the resulting solution was then filtered through a 0.45 µm membrane filter, followed by the addition of mobile phase to the 50 mL mark to obtain a stock solution (1 mg/mL). The stock solution was appropriately diluted with mobile phase to obtain the suitable working concentration (80 µg/mL). The working standard solution was injected into the HPLC system thrice. The concentration of the RXN in the tablets was calculated using the calibration curve or from the regression equation.

Analysis of Placebo blank solution

An accurately weighed amount of starch (10 mg), acacia (10 mg), hydroxyl cellulose (10 mg), sodium citrate (10 mg), talc (10 mg), magnesium stearate (10 mg), lactose (10 mg), glucose (10 mg) and sodium alginate (10 mg) were transferred into a 50 mL volumetric flask containing 30 mL of mobile phase. This mixture was sonicated for 15 minutes, filtered and followed by the addition of mobile phase to the 50 mL mark. This solution was injected into the HPLC system.

Procedure for forced degradation studies

Acid and alkali degradation

In order to perform acid and alkaline degradation, 100 mg of RXN was mixed with 10 mL of 0.1 N HCl and 0.1 N NaOH in 100 mL volumetric flasks, respectively. The solutions were allowed to react for 2 hours at 80ºC temperature in the water bath. After that, the solutions were neutralized with 0.1 N HCl (alkaline degradation), 0.1 N NaOH (acid degradation) and then diluted with mobile phase up to the mark.

Oxidative degradation

To promote the oxidation, 100 mg of RXN was mixed with 10 mL of 3% H_2O_2 in 100 mL volumetric flask and left to react for 2 hours at 80 ºC temperature in the water bath. After oxidation, the solution was diluted with mobile phase up to the mark.

Dry heat degradation

For this purpose, 100 mg of RXN was taken in glass petri dish and kept in a hot air oven (105 °C) for 2 hours. After the specified time, the sample was transferred to a 100 mL volumetric flask containing 30 mL of mobile phase, mixed well and diluted up to the mark with mobile phase.

Photolytic degradation

For photolytic degradation, 100 mg of RXN was taken in a glass petri dish and exposed to sunlight for 24 h. After the degradation, the sample was transferred to a 100 mL volumetric flask containing 30 mL of mobile phase and mixed well. The volume of the flask was completed up to mark with mobile phase.

The above degraded sample solutions were filtered and appropriately diluted with mobile phase to 80 µg/mL of RXN. The degraded sample solutions were injected into the HPLC system. The respective chromatograms and peak area were recorded.

Results and Discussion

Method development

During the method development two columns [thermo BDS hypersil-C8 (250 mm x 4.6 mm x 5 µm) and ACE-Ciano column (250 mm x 4.6 mm, 5 µm particle size)], different pH values (3-7)

and two organic solvents (methanol and acetonitrile) were tested. Acceptable separation, peak shape and symmetry, with a retention time of 4.462 minutes, were achieved with ACE-Ciano column (250 mm x 4.6 mm, 5 µm particle size), mobile phase consisting of 0.1 M Sodium acetate (pH 5.5): methanol (60:40, *v/v*) at 1 mL/min flow rate and 30 °C temperature. The RXN solution in mobile phase was scanned by UV-visible spectrophotometer. It was observed that the maximum absorbance of RXN was obtained at 247 nm.

Method validation

System suitability

For this purpose, five replicate injections of freshly prepared standard solutions (80 µg/mL) were made. Percentage relative standard deviation of the system suitability parameters was calculated. The low percentage relative standard deviation values (Table 1) indicate that all the system suitability parameters are within the acceptable limits.

Table 1. System suitability

Parameter	Mean value (n=5)	% RSD
Retention Time (min)	4.469	0.136
Peak area	13376045	0.289
Theoretical Plates (n)	5232	1.325
Plates per Meter (N)	21292	1.326
Height equivalent to theoretical plate (HETP)(mm)	4.696×10^5	1.320
Tailing factor	1.259	0.258

Linearity

Linearity was found in the concentration range 1-120 µg/mL RXN. The regression equation was: A = 167925 x – 99.638 (where A = peak area, and x = concentration of RXN in µg/mL) with regression coefficient (R^2) of 0.9992.

Sensitivity (LOD and LOQ)

For the determination of LOD and LOQ, five replicate injections of dilute solutions with known concentration (1 µg/mL) were injected into the HPLC system. The LOD and LOQ of the method are found to be 0.194 µg/mL and 0.648 µg/mL, respectively. These values indicate the excellent sensitivity of the proposed method.

Precision and Accuracy

The intra- and inter-day precision and accuracy of the method was carried out at three different concentration levels (1 µg/mL, 80 µg/mL and 120 µg/mL). The precision and accuracy was expressed in terms of percentage relative standard deviation (%RSD) and percentage recovery, respectively. The low %RSD (<1) and good percentage recovery values showed that the precision and accuracy of the method was good (Table 2).

Recovery studies via standard addition method

The accuracy of the proposed method was further assessed by carrying out recovery studies through standard addition method. The preanalyzed tablet samples were spiked with 50%, 100% and 150% of RXN found in tablet sample. The percent recovery values (Table 3) indicate that the recovery was good and the excipients present in the tablet dosage form did not interfere in the assay of RXN.

Table 2. Precision and accuracy

Type of analysis	Concentration of RXN (µg/mL)		Precision	Accuracy	
	Taken	Found (n=5)	%RSD	% Recovery	% Error
Intra-day analysis	1	1.001	0.579	100.100	0.100
	80	79.946	0.268	99.932	0.068
	120	119.858	0.112	99.881	0.119
Inter-day analysis	1	1.004	0.298	100.400	0.400
	80	79.995	0.322	99.993	0.007
	120	120.145	0.064	100.120	0.120

Table 3. Quantification of RXN by standard addition method

Concentration of RXN, mg		%	%
In tablet + Spiked	Found (n=3)	RSD	Recovery
10 + 5	14.931	0.314	99.540
10 + 10	19.932	0321	99.660
10+ 15	24.950	0.156	99.980

Selectivity

The selectivity of the method was evaluated through the comparison of the chromatograms of placebo blank solution, blank mobile phase and tablet sample solution with the standard drug solution (80 µg/mL). There is no peak interference of mobile phase blank, placebo blank and tablet excipients at the retention time of RXN (Figure 1). The results established the selectivity of the method.

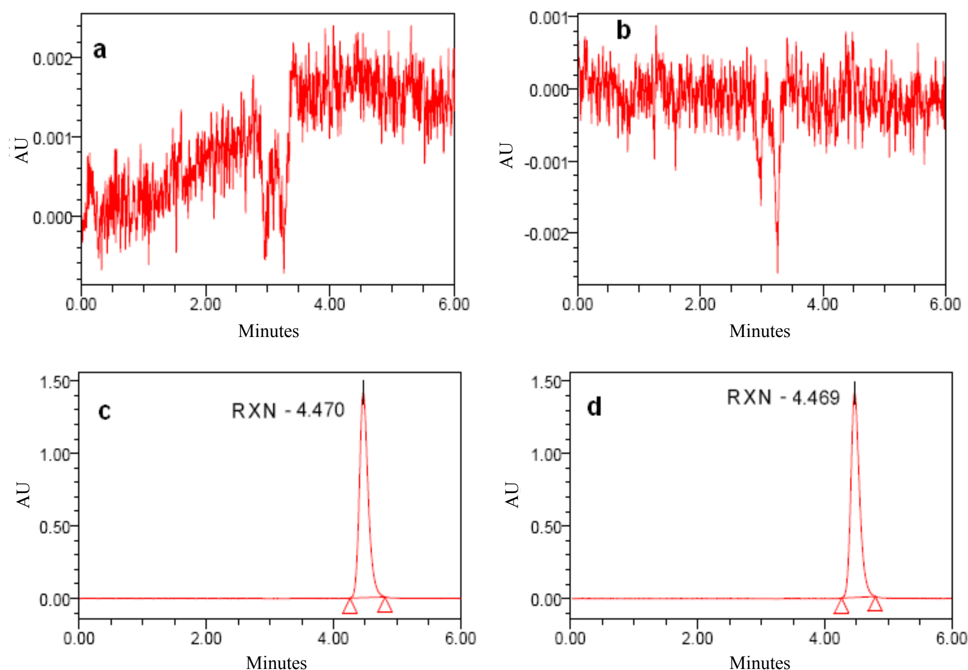

Figure 1. Chromatogram of (a) Mobile phase blank (b) Placebo blank (c) Pure RXN (d) RXN tablet sample

Specificity

In order to evaluate the specificity of the proposed method, forced degradation studies were performed. The samples submitted to acid and alkaline conditions, RXN content had a decrease of 7.705% and 8.851%, respectively. When the oxidation with H_2O_2 was promoted, the RXN content had a decrease of 6.559%. When the RXN powder was exposed to dry heat at 105°C for 2 hours, 9.186% of RXN degradation was observed. 7.304% of RXN degradation was observed after exposure of solid drug to sunlight for 24 hours. The chromatograms recorded for the degradation samples revealed no peaks within retention time of RXN (Figure 2). The good resolution of RXN peak and the degradation product in all the situations could be observed, indicating the specificity of the method.

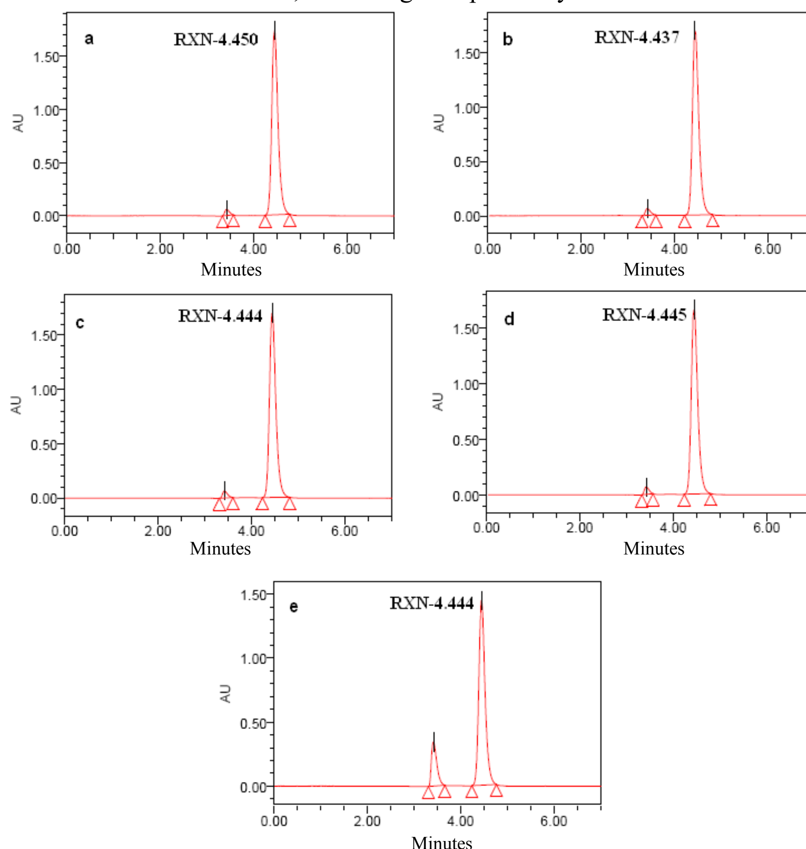

Figure 2. Chromatogram of RXN after (a) Acid degradation (b) Alkali degradation (c) Oxidative degradation (d) Dry heat degradation (e) Photolytic degradation

Robustness

The robustness of the method was determined by analyzing the RXN under a variety of conditions of the method parameters, such as the mobile phase ratio, pH of the mobile phase, flow rate and column temperature. The low %RSD values (<1) (Table 4) indicate that there were no significant changes in the peak area when deliberate modifications were made in the experimental conditions, consequently showing the method to be robust.

Table 4. Robustness

Experimental variable	Investigated range	1 µg/mL RXN		120 µg/mL RXN	
		Mean Peak area (n=3)	%RSD	Mean Peak area (n=3)	%RSD
Mobile phase ratio* (*v/v*)	58:42 60:40 62:38	166975	0.234	20017035	0.290
pH of mobile phase	5.1 5.2 5.3	166823	0.348	20005713	0.104
Temperature of the column (°C)	28 30 32	165607	1.024	20037150	0.576
Flow rate of mobile phase (mL/min)	0.9 1.0 1.1	170356	0.363	20033122	0.412

**mobile phase composition: 0.1 M sodium acetate and methanol*

Application to tablet dosage forms

The tablet formulations containing RXN were analyzed using the developed HPLC method. The percent recovery was 99.74%, while the RSD value was 0.421%. The recovery value indicates the non interference of commonly added tablet excipients with the assay.

Comparison of the proposed method with the reported methods

In comparison with the earlier reported methods for the quantification of RXN (Table 5), the proposed method has the advantages such as broad linearity range[6-9,16], more precise[3-6,13,14], more accurate[3-6,8,11,13,14] and sensitive[5-7]. Unlike the bioanalytical methods[8-12], the proposed method does not require cumbersome extraction procedure, internal standard, costly detector system and expertise operational personnel. The reported RP-HPLC and stability indicating HPLC methods used acetonitrile in their mobile phase which probably will raise the cost of the method. In all the reported methods, except the HPLC method reported by Çelebier *et al.*[13] forced degradation studies were not reported. Some of the methods[4,8-12,14] were not applied to tablet formulations.

Table 5. Comparison between proposed and reported methods

Method	Linearity µg/mL	RSD %	LOQ µg/mL	Recovery %	Reference
Visible spectrophotometry	2-12	0.47	0.1	98.17	[5]
Visible spectrophotometry	3-21	0.88	0.5	98.58	[5]
Visible spectrophotometry	30-90	0.94	10	98.53	[5]
Visible spectrophotometry	5-30	0.52	0.5	99.30	[5]
Visible spectrophotometry	15-90	1.02	5	99.25	[5]

Contd…

UV spectrophotometry	2-12	0.125-0.915	0.298	100.85	[3]
UV spectrophotometry	2-12	0.205-1.076	0.642	99.90-100.50	[4]
RP-HPLC	40-100	0.56-0.66	6	98.8	[6]
RP-HPLC	50-200	0.471	2.47	99.708	[7]
Stability indicating HPLC	5-40	1.17	0.005	100.94	[13]
Stability indicating HPLC	20-100	0.147	0.387	100.85	[14]
HPLC-MS/MS	0.5-500	7.4*	-	96.3-102.90	[8]
Anti-factor Xa chromogenic assay	0-433.3**	-	-	-	[9]
Anti-factor Xa chromogenic assay	-	7-8.8*	-	-	[10]
Anti-factor Xa chromogenic assay	20-660**	10 -19.1*	-	90-99.09	[11]
Anti-factor Xa chromogenic assay	25-90**	2.02 -5.43*	-	-	[12]
Stability indicating HPLC	1-120	0.064 - 0.579	0.648	99.74	Proposed method

**coefficient of variation, ** ng/mL*

Conclusion

A new stability indicating HPLC method was developed and validated for the determination of rivaroxiban. The ICH guideline was adhered for method validation. Satisfactory sensitivity, precision, accuracy, selectivity, specificity and minimum cost are the main features of the developed method. The present study demonstrates the degradation vulnerability of rivaroxiban to different stress conditions. The method was proved adequate for quantitative determination of rivaroxiban in presence of its degradation products. The method was found to be suitable for the analysis of rivaroxiban in tablet dosage form.

Acknowledgement

One of the authors, BSV Seshamamba, is thankful to the Department of Food Chemistry and Nutrition, College of Food Science and Technology, Bapatla for their continuous support & encouragement and Rainbow Pharma Training Lab, Hyderabad for providing the necessary facilities to carry out the work.

References

1. Piccini J P, Patel M R, Mahaffey K W, Fox K A and Califf R M, *Expert Opin Investig Drugs*, 2008, **17(6),** 925-937; DOI:10.1517/13543784.17.6.925
2. Alban S, *Curr Pharm Des*., 2008, **14(12),** 1152-1175.
3. Muralikrishna K S and Kasad P A, *Inventi Rapid: Pharm Anal Qual Assur*., 2013, Article ID-Inventi:ppaqa/699/13.
4. Sekaran C B, Bind V H, Damayanthi M R and Sireesha A, *Der Pharma Chemica.,* 2013, **5(4)**, 1-5.
5. Satyanarayana P V V and Madhavi A S, *Int J Res Rev Pharma Appl Sci*., 2012, **2(4)**, 611-620.

6. Satyanarayana P V V and Madhavi AS, *Int J Sci Inno Dis*., 2012, **2(1)**, 226-231.
7. Sekhar K C, Vani P S, Lakshmi A D, Devi Ch L L, Anupama B and Narendra D, *Research Desk*., 2012, **1(1)**, 24-33.
8. Rohde G, *J Chromatogr B Analyt Technol Biomed Life Sci*., 2008, **872(1-2)**, 43-50.
9. Mani H, Rohde G, Stratmann G, Hesse C, Herth N, Schwers S, Perzborn E and Lindhoff-Last E, *Thromb Haemost*., 2012, **108(1)**, 191-198.
10. Asmis L M, Alberio L, Angelillo-Scherrer A, Korte W, Mendez A, Reber G, Seifert B, Stricker H, Tsakiris D A and Wuillemin W A, *Throm Res*., 2012, **129(4)**, 492-498; DOI:10.1016/j.thromres.2011.06.031
11. Samama M M, Contant G, Spiro T E, Perzborn E, Guinet C, Gourmelin Y, Le Flem L, Rohde G and Martinoli J L, *Thromb Haemost.*, 2012, **107(2)**, 379-387.
12. Harenberg J, Roland K, Christina G, Svetlana M, Christel W and Martin W, *J Thromb Thrombolysis*, 2011, **32(3)**, 267–271; DOI:10.1007/s11239-011-0622-5
13. Celebier M, Recber T, Kocak Engin and Altinoz S, *Braz J Pharm Sci*., 2013, **49(2)**, 359-366.
14. Kasad P A and Muralikrishna K S, *Asian J Pharm Anal*., 2013, **3(2),** 62-65.
15. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use Guidelines, Q1A(R2): Stability Testing of New Drug Substances and Products. 2003, 1-24.
16. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use Guidelines, Q2(R1): Validation of Analytical Procedures. 2005, 1-17.