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DEVELOPMENT AND VALIDATION OF BIOANALYTICAL METHOD FOR ESTIMATION OF RIVAROXABAN USING HPLC-PDA IN HUMAN BLOOD PLASMA

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ABSTRACT

Rivaroxaban is oral anti-coagulant drug, used in the treatment of various blood disorders. A sensitive and accurate liquid chromatographic methodfor the quantification of rivaroxaban in human plasma was successfully developed. Protein precipitation was used for sample preparation. Quantification of analyte was achieved on phenomenexluna C_8 (5µm x 25cm x 4.6mm i.d.) column using methanol:water:dimethyl sulfoxide (50:45:5, v/v/v) as mobile phase at a flow rate of 1ml/min. Detection was achieved at 252nm over the linearity concentration range of 5-40µg/ml. Retention time was found 6.2 min. The LOD and LOQ were found to be 1.5 µg/ml and 4.5 µg/ml, respectively.The developed method was validate as per US FDA guidelines.

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INTRODUCTION:

Anticoagulants are given to prevent the blood clotting or to prevent existing clots from getting larger. Clots can block the blood flow to the brain or to the heart muscle.These blockages may cause a heart attack or a stroke.Rivaroxaban (RIV)is potent and effective oral anti-coagulant drug, which is widely used in the treatment of thromboembolic ailments, myocardial infarction, stroke, angina pectoris and various blood clotting related disorders. IUPAC name of RIV is (S)-5-chloro-N-{[2-oxo-3-[4-(3-oxomorpholin-4-yl) phenyl] oxazolidin-5-yl]methyl}thiophene-2-carboxamide (Fig.1).RIV is a small molecule(molecular weight 436 $gmol^{-1}$) and almost insoluble in water. It is rapidly absorbed through gastro intestinal tract and attains maximum plasma concentrations at 2–4 h after oral administration. Oral bioavailability of RIV is high (92–98 %) with the dose of 10 mg, irrespective of food intake. RIV acts as anticoagulant by inhibiting the factor Xa. Inhibiting factor Xa,it activates the generation of thrombin molecules.

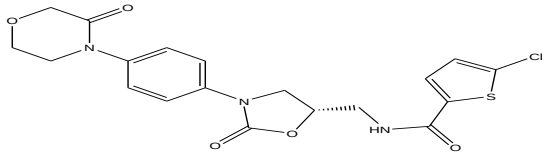


Figure 1: Chemical structure of Rivaroxaban

The factor Xa inhibition occurs mainly as a result of RIV binding with S1 and S4 pockets of the serine endopeptidaseenzyme with high selectivity.

The literature review revealed that there are few bioanalytical methods reported for the determination of rivaroxaban in human blood plasma[1-5]. There is only one HPLC-UV method till date for RIV quantification in human plasma[3]. The sample preparation in above reported method is very tedious, further above method utilized acetonitrile and water as mobile phase in the ratio 55:45 % v/v. Method requires quite high concentration of acetonitrile. However, present studies attempts to develop simplified and eco-friendly sample preparation procedure and mobile phase to implement greener approach for the quantification of RIV in human plasma.

MATERIAL AND METHODS:

Materials Rivaroxaban was procured as a gratis sample from Mehta API Pvt. Ltd., Mumbai, India. Chloramphenicol was procured from Panchsheel organics, Indore, India. HPLC grade water was procured from Medicaps Pvt. Ltd. Dhar, India. Methanol, acetonitrile and dimethyl sulfoxide (DMSO) were purchased from spectrochem Pvt. Ltd, Mumbai, India. Human plasma wascollected from Greater Kailash Hospital, Indore, India. The required chromatographic solvents and solutions were first filtered through

0.45µmmembrane filters (Pall life sciences, India) and sonicated prior to use.

Method *Preparation of standard solutions:* Stock solutions of RIV (1000µg/ml) and Chloramphenicol, internal standard (IS) (1000µg/ml) were prepared in DMSO. Serial dilutions of stock solutions were prepared and used as working standard for analysis.

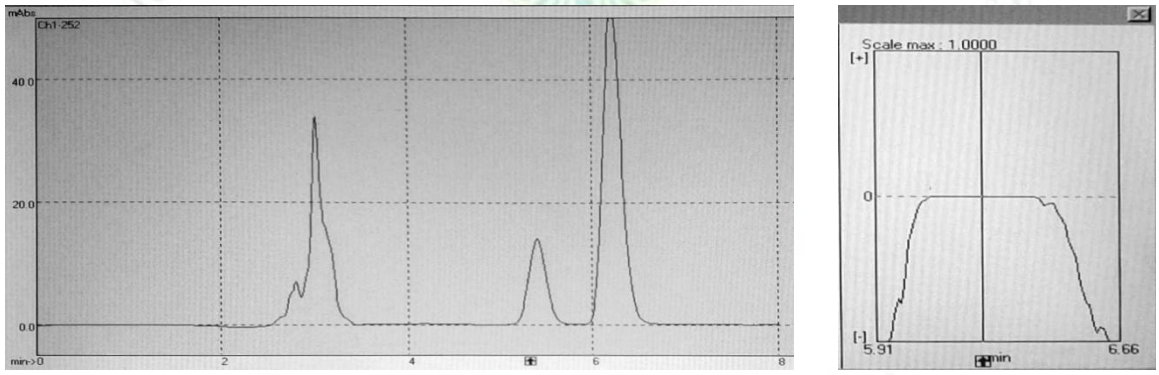
Sample preparation: Human plasma (0.1ml) was taken in a 2ml poly propylene tube, 20µl of standard drug and IS were spiked into it, then 0.5 ml of acetonitrile was added. These solutions were vortexed for 1 min and then centrifuged for 10 min at 10000 rpm, supernatant was injected to chromatographic system. Sample preparation was done in triplicate.

Chromatographic conditions: The mobile phase consisted methanol: water: DMSO (50:45:5, % v/v/v) at a flow rate of 1.0 ml/min. The required chromatographic solvents and solutions were first filtered through 0.45 µm membrane filters (Pall life sciences, India) and sonicated prior to use. Chromatographic separation was achieved at ambient temperature on HPLC system having a pump (Shimadzu LC $10AT_{VP}$) with 20 µL Rheodyne injector, Phenomenex Luna C_8 (5 µm x 25cm x 4.6mm i.d) column and SPD-10 A_{VP} photodiode array (PDA) UV-Visible detector set at 252nm and equipped with *CLASS-VP* software (Shimadzu, Japan). The run time was kept 8 min. Calibration curve is in the range of 5 µg/ml to 40 µg/ml. Peak purity is checked by obtaining ratio chromatogram.

Sample analysis: The responses of sample solutions were measured at 252 nm for quantitation of RIV by using the HPLC method as described above. The amounts of RIV present in sample solution were determined by applying value of peak area to the regression equation of the calibration graph. Validation was done as per US FDA guidelines. The specificity of the developed method was determined by comparing test results obtained from RIV solution containing plasma with that of results obtained from standard RIV solution. The linearity equation was obtained by linear regression analysis method by plotting a graph between mean peak response area and concentration. LOD and LOD were determined using dilution method. The recovery experiments were performed by the standard addition method.

RESULTS AND DISCUSSION:

The literature revealed that RIV is insoluble in water and less soluble in various solvents. DMSO was used as solvent for RIV. Several trials were performed to remove the interferences of biological matter in the sample. Final sample preparation involves precipitation of undesirable matrix component by protein precipitation using acetonitrile.



A B

Figure 2: RP-HPLC Chromatogram of Rivaroxaban with IS in human plasma

To optimize the HPLC parameters, several mobile phase compositions were tried.

Table 1: System suitability test for the proposed HPLC method

Parameters	Rivaroxaban ± % RSD[a]
Retention time (R_t), min	6.2 ± 0.03
Tailing factor	1.16 ± 0.04
Resolution (Rs)	2.19 ± 0.04
Theoretical plates (N)	4249 ± 0.8

[a]% Relative standard deviation, (n=6)

A satisfactory separation of RIV with good peak symmetry and steady baseline was obtained with the mobile phase methanol: water: DMSO (50:45:5, %v/v/v) at a flow rate of 1.0 ml/min. Quantitation was achieved with UV detection at 252nm based on peak area. Complete resolution of the peak with clear baseline separation was obtained (Fig. 2). The system suitability test parameters are shown in Table 1.

Method validation parameters:

System suitability: System suitability parameters were found to be satisfactory. All the parameters, theoretical plate count (N) >4100, resolution (Rs) >2 and tailing factor >1.2 were within acceptable value. The relative standard deviation (RSD) of peak area was found to be <2percentage.

Specificity: There is no chemical interaction between the RIV and IS, and both the RIV and IS was resolved well. There were no interfering peak/s found in the chromatogram obtained from the blank plasma at the retention times of RIV and IS.

Linearity: The linear regression correlation coefficient of 0.975 was obtained over the six different concentration levels ranging from5 µg/ml to 40 µg/ml for RIV. The average slope and intercept of linearity equations were 26709.03 and -21174.56 respectively. Regression analysis data are summarized in Table 2.

Table 2: Regression analysis of calibration graphs for RIV for the proposed HPLC methods

Parameters	HPLC
Conc. Range	5-40 µg/ml
Slope	26709.03
SD of slope	110.98
Intercept	-21174.56
SD of intercept	1154.79
correlation	0.9735
Regression equation	y= 26709x-21174

Y = peak area
x = Concentration of analyte in µg/ml

Limit of detection (LOD) and limit of quantitation (LOQ): The concentration of RIV for determination of LOD was 1.5 µg/ml, which indicates the sensitivity of the method. Similarly, LOQ was found 4.5 µg/ml, which proves that RIV can be estimated at low concentration.

Accuracy and Precision: The interday and intraday precision values of RIV for various concentrations ranged from 0.57% to 1.15% RSD and0.46% to 0.88% RSD, respectively. The values for accuracy were also found within acceptable limits at the same concentrations. The data are presented in Table 3.

Table 3: Method validation parameters for estimation of Rivaroxaban

Parameters	HPLC
Limit of detection	1.5 µg/ml
Limit of quantification	4.5 µg/ml
Recovery	99.38-100.44%
Repeatability (%RSD, n=6)	0.64
Intermediate Precision	
Interday (%RSD, n=3)	0.57-1.15
Intraday (%RSD, n=3)	0.46-0.88
Robustness (%RSD)	< 2%

[a]%RSD = Relative standard deviation

Robustness: The developed method was found to be robust during robustness studies, the %RSD was found to be <2 in each case. The low values of %RSD showed the robustness of the method (Table 3).

Sample and standard stability: The stability of RIV sample in human plasma and the standard solutions were determined. Peak area and retention time variation were found to be <1%. Also, no significant change in peak area was observed during 24 h.

CONCLUSIONS:

In the present study, an attempt was made to develop a simple, precise, selective and sensitive validated bioanalytical method of RIV using RP-HPLC. This method is quite simple, economic, less time-consuming method up to date for the determination of RIV in plasma with RP-HPLC.

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REFERENCES:

1. Rohde G, Determination of rivaroxaban--a novel, oral, direct Factor Xa inhibitor--in human plasma by high-performance liquid chromatography-tandem mass spectrometry, J Chromatogr B, 2008, 872, 43-50.
2. Kuhn J, Gripp T, Flieder T, Dittrich M, Hendig D, Busse J, UPLC-MRM Mass Spectrometry Method for Measurement of the Coagulation Inhibitors Dabigatran and Rivaroxaban in Human Plasma and its Comparison with Functional Assays, PLoS ONE, 2015, 10 (12),78.
3. Celebier M, Reçber T, Koçak E, Altınöz, Kır S, Determination of Rivaroxaban in Human Plasma by Solid-Phase Extraction–High Performance Liquid Chromatography, J Chrom Sci, 2015, 7, 1-5.
4. Zhang W, Lou D, Zhang DT, Zhang Y, Huang HJ, Determination of rivaroxaban, apixaban and edoxaban in rat plasma by UPLC–MS/MS method, J Thromb Thrombolysis, 2016, 1-7.
5. Reddy GS, Prasad RSLN, Reddy LSK, Development and Validation of Hplc-Ms/Ms Method for Rivaroxaban Quantitation in Human Plasma Using Solid Phase Extraction Procedure, Orient J Chem, 2016, 32 (2), 1145-1154.