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Original Article

ENANTIOMERIC SEPARATION OF RIVAROXABAN BY A CHIRAL LIQUID CHROMATOGRAPHIC METHOD

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ABSTRACT

Objective: To develop a novel, simple, selective and enantiomeric separation of rivaroxaban by a chiral liquid chromatographic method as per ICH guidelines.

Methods: An enantioselective reversed phase high performance liquid chromatographic method was developed and validated. The enantiomers of rivaroxaban was resolved on a Chiralcel OD-H (250 mm × 4.6 mm, 5 mm) column using a mobile phase system containing n-hexane –isopropanol (50: 50 v/v/) and column temperature at 35°C. The resolution between the enantiomers was not less than 2.0. The developed method was validated according to ICH guidelines.

Results: The calibration curve was found to be linear over the concentration range of 0.075–1.2µg/mL (r^2= 0.9996). The limit of detection and limit of quantification of the (R)-enantiomer were found to be 0.025 and 0.075µg/mL, respectively, for 20 mL injection volume. The percentage recovery of the (R)-enantiomer ranged from 92.06 to 105.9 in bulk drug samples of rivaroxaban. The final optimized method was successfully applied to separate the (R)-enantiomer from rivaroxaban and was proved to be reproducible, accurate and robust for the quantitative determination of the (R)-enantiomer in Rivaroxaban.

Conclusion: A novel, simple, selective and simple, selective and enantiomeric separation of rivaroxaban by a chiral liquid chromatographic method was developed as per ICH guidelines.

Hence, the method can be used for routine analysis in pharmaceutical industry.

Keywords: HPLC, Rivaroxaban, Chiral, Enantiomeric separation.

INTRODUCTION

Rivaroxaban is an oral anticoagulant inventedand manufactured by Bayer; it is marketed as Xarelto [1] and shown in (Figure1). Its molecular weight is 435.89 and molecular formula is $C_{19}H_{18}ClN_3O_5S$ [2]. It is an oxazolidinone derivative anticoagulant that inhibits both free Factor Xa and Factor Xa bound in the prothrombinase complex [3].

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 [4].

A thorough literature search has revealed that high performance liquid chromatography (HPLC), liquid chromatography-mass spectroscopy (HPLC-MS), high performance thin layer chromatography (HPTLC), UV spectrophometry (UV) methods have been reported for determination of rivaroxaban in tablet dosage form as pharmaceutical formulation, and plasma [5-8]. During the synthesis of rivaroxaban there is a possibility of carrying an undesired(R)-enantiomer of enantiopure rivaroxaban, therefore enantioselective analytical methods are necessary to ensure its therapeutic efficacy and safety. However, the development of methods for the quantitative analysis of chiral compounds and for the assessment of enantiomeric purity is extremely challenging, because the same physical and chemical properties of the two enantiomers make discriminating and separating them very difficult.

This paper describes a Novel Chiral HPLC method for the Enantiomeric separation of rivaroxaban enantiomers using cellulose based chiral stationary phase, Chiralcel OD-H. The aim of this work was to optimize the chromatographic conditions in terms of temperature, flow rate, in order to separate and identify the enantiomers of rivaroxaban. The developed method was validated according to International Conference on harmonization (ICH) guidelines [9] for the quantitative determination of the (R)-enantiomer in Rivaroxaban.

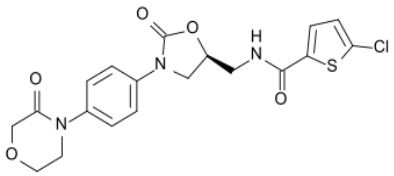

Fig. 1: Rivaroxaban structure

Equipment

HPLC system used was Shimadzu (LC 2010 series, Japan) system equipped with auto sampler, quaternary pump, degasser, and a UV Detector. The out put signal was monitored and processed using Lab solutions software.

Sample preparation

The stock solution of the (*R*)-enantiomer and rivaroxaban (1.0 mg/mL) was prepared by and adding first 0.2 ml of dimethylsulfoxide and then dissolving an appropriate amount of the substance in mobile phase. For quantitation of (*R*)-enantiomer in rivaroxaban, a solution of 0.75 µg/mL concentration was used.

Chromatographic condition

The chromatographic column used was 250 × 4.6 mm,5 µ, Chiral Pak OD-H (Daicel Chemical Industries, Ltd., Tokyo, Japan) packed with 5 µm particles. The mobile phase was n-hexane–ethanol (50:50, v/v). The flow rate of the mobile phase was 1.0 mL/min. The column temperature was maintained at 35°C, and the eluent was monitored at a wavelength of 250 nm. The injection volume was 20 µL. Amylose

based chiral stationary phase AD-H, modified cellulose based Chiralcel OD-RH, and cellulose based chiral stationary phase Chiralcel OJ-H(Diacel Chemical Industries, Ltd., Tokyo, Japan) was employed during the method development.

Method validation

System suitability

The system suitability was determined by injecting a mixture containing 0.15% (w/w) of (*R*)-enantiomer and rivaroxaban (0.5 mg/ml). Because the enantiomers form a critical band pair in the chromatogram, the qualification criteria were the resolution between the two enantiomers, shown to be not less than 1.5 and tailing factor should not exceed 1.5. The separation factor (α) was calculated as the ratio of retention factors, α = k2/k1. The resolution factor (Rs) was calculated as Rs = 2(t2 – t1)/(w1 + w2) where, t1, t2 refer to the retention time of the first and second enantiomers; w1 and w2 are the peak widths for the first and second eluting enantiomers, respectively.

Precision

Method reproducibility was determined by measuring repeatability retention times and peak areas for each enantiomer. In order to determine the repeatability of the method, replicate injections (n = 6) of a 0.5mg/mL solution containing Rivaroxaban spiked with (*R*)-enantiomer (0.15%) were carried out.

Linearity of (*R*)-enantiomer

Linearity was assessed by preparing six calibration sample solutions of (*R*)-enantiomer covering from 25µg/mL (LOQ) to 150µg/mL (25, 50, 80, 100,120 and 150µg/mL), preparedin mobile phase from (*R*)-enantiomer stock solution. The regression curve was obtained by plotting peak area versus concentration, using the least squares method. The percentage relative standard deviation of the slope and Y-intercept of the calibration curve was calculated.

Recovery of (*R*)-enantiomer

The study was carried out in triplicate at 50, 100, and 150µg/mL of the rivaroxaban target analyte concentration. The recovery of (*R*)-enantiomer was calculated from the slope, and Intercept of the calibration curve obtained was ensured by determining recovery of the spiked amount of (*R*)-enantiomer in rivaroxaban.

LOD and LOQ of (*R*)-enantiomer

LOD and LOQ of (*R*)-enantiomer were achieved by injecting a series of dilute solutions of (*R*)-enantiomer. The precision of the developed enantioselective method for (*R*)-enantiomer at LOQ was checked by analyzing six test solutions prepared at the LOQ level and calculating the percentage relative standard deviation of area.

Robustness

To determine robustness of the method, experimental conditions were purposely altered, and chromatographic resolution between enantiomers was evaluated. The flow rate of the mobile phase was 1.0 mL/min. To study the effect of the flow rate on the resolution of enantiomers, it was changed 0.1 units from 0.9 to 1.1 mL/min, while the other mobile phase components were held constant. The effect of column temperature on the resolution was studied at 30°C and 40°C while other mobile phase components were held constant.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

A racemic mixture solution of rivaroxaban and its (R)-enantiomer (1 mg/mL each) prepared by adding 0.2 ml dimethylsulfoxide and then dissolving in the mobile phase for method establishment. To develop a rugged and suitable HPLC method for the separation of the two enantiomers, different stationary phases and mobile phases were employed. Preliminary column screening involved Chiralpak OD-RH (150 mm×4.6 mm, 5 mm) and Chiralcel OJ-H (150 mm×4.6 mm, 5 mm),Chiralpak AD-H (250 mm×4.6 mm, 5 mm) of Daicel were also employed. Only Chiralpak OJ-H [Cellulose tris (4-methylbenzoate)] andChiralcel OD-H [cellulose tris-(3,5-dimethylphenylcarbamate)] columns provided selectivity between the rivaroxaban peak and the undesired enantiomer peak using a mobile phase consisting of n-hexane – ethanol (70: 30, v/v), but the retention times of rivaroxaban and (R)-enantiomer were both longer than 30 min and the peaks were broad. We continued to select the best mobile phases that would give optimum resolution and selectivity for the two enantiomers. Good separation was achieved on Chiralcel OD-H and Chiralpak OJ-H columns n-hexane–isopropanol (50:50 v/v)as the mobile phase. There was no separation when phosphate bufferof (pH 4.0) and methanol used as mobile phase on Chiralcel OD-RH.

In another attempt, buffer was removed and only Water: Acetonitrile and 100% Acetonitrile as a mobile phase were tried on Chiralcel OD-RH column, but there was no separation.

While on Chiralcel AD-H column, n-hexane and ethanol mixture and n-hexane-isopropanol mixrture used as mobile phase to check enantiomeric separation. But it failed to resolve enantiomers. Similarly, attempts were made to increase the flow rate to 1.5 ml/min so that it will reduce the run time,but resolution was very poor and not satisfactory and foundless than 1.5.

Due to the better chromatographic results obtained on the Chiralcel OD-H column, further method optimization andquantification of the (R)-enantiomer were carried out on this column. Based on the data obtained from method developmentand optimization activities, the Chiralcel OD-H (250 mm×4.6 mm, 5 mm) column with the mobile phase of n-hexane–isopropanol (50:50 v/v) was selected for the final method. The flow rate of the experimental method was 1.0 mL/min with an injection volume of 20 mL. The column temperature was 35°C, and the detection wavelength was 250 nm. Under these conditions, the two enantiomers were separated well and the peak of the (R)-enantiomer eluted before the peak of rivaroxaban (S-isomer). In the optimized method, the typical retention times of the (R)-enantiomer and rivaroxaban (S-isomer) were approximately 16 and24 min, respectively. Baseline separation of rivaroxaban and(R)-enantiomer was obtained with a total run time of 30 min. The chromatographic separation of rivaroxabanenantiomers under the optimized conditions using an ultraviolet detector (UV) detector on Chiralcel OD-H column is shown in (fig. 2, 3 and 4)

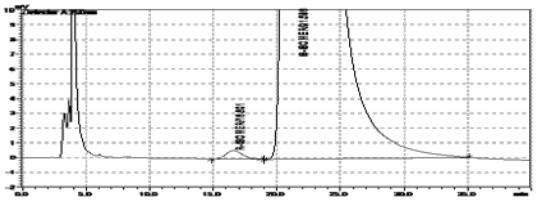

Fig. 2: System suitability chromatogram (R-isomer Spiked at 100% level + S-isomer of 500 ppm)

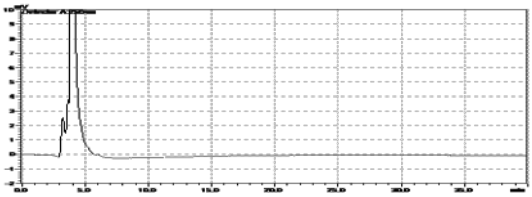

Fig. 3: Blank chromatogram

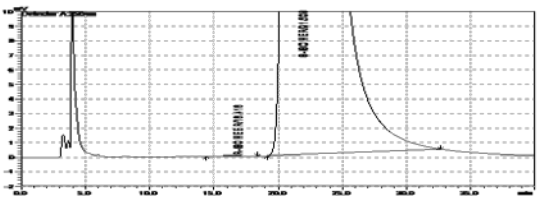

Fig. 4: Test chromatogram of Rivaroxaban

Validation of the method

System suitability

The system suitability solution was run and the resolution was found more than 1.5 within isomers and tailing factor was not more than 2.0 (Table 1)

Table 1: System suitability criteria

Enantiomer	Retention time (min)	Relative retention time (min)	Resolution	Tailing factor
(*R*)-enantiomer	16.4	0.7	2.0	1.1
(*S*)-enantiomer	22.4	1.0	---	1.8

Method reproducibility

Method reproducibility was determined by measuring retention times and peak areas for each enantiomer. The repeatability was expressed as relative standard deviation (R. S. D.). For this study, rivaroxaban (0.5 mg/mL) spiked with the (R)-enantiomer (0.15% w/w) was analyzed in six replicates to establish repeatability.

Limit of detection and limit of quantification of (R)-enantiomer

The LOD and LOQ were determined for (R)-enantiomer based on the percentage relative standard deviation of (%RSD) of the response precision. Concentrations of LODs were found 0.025 µg/ml while LOQ was found 0.075µg/ml for (R)-enantiomer (Table 2).

Linearity of (R)-enantiomer

The percentage relative standard deviation of the slope and Y-intercept of the calibration curve was calculated. Linearity of the (R)-enantiomer was evaluated over seven levels of (R)-enantiomer solutions from 0.075 (LOQ level) to1.2 µg/mL (150% level). (Table 2).

Recovery study

The standard addition and recovery experiments were conducted for the (R)-enantiomer in bulk samples in triplicate at50% (0.35µg/mL), 100% (0.75µg/mL) and 150% (1.20µg/mL)with respect to test concentration. A HPLC chromatogram of the rivaroxaban bulk drug sample (0.5 mg /mL) spiked with the (R)-enantiomer (0.15%) is shown in Fig. 2. The percentage recovery ranged from 92.09% to 105.99 % (Table 3).

Table 2: Linearity, LOD, LOQ and recovery results

Validation parameter	Results
Repeatability (n = 6, % RSD)	
Retention time (*R*-enantiomer)	16.7
Peak area (*R*-enantiomer)	4865
LOD-LOQ (R-enantiomer)	
LOD (µg/mL)	0.025
LOQ (µg/mL)	0.079
Precision at LOQ (%RSD	4.0
Linearity (R-enantiomer)	
Calibration range (µg/mL)	0.07 to 1.20
Slope	71029.15
Intercept	-387.68

Table 3: Recovery results of (*R*)-Enantiomer in bulk sample

Level	Recovery (%)
50%	98.0
100%	92.09
150%	105.99

Table 4: Results from evaluation of the robustness parameter for (*R*)-Enantiomer

	Retention time of (*R*)-Enantiomer (min)	Resolution between Rivaroxaban and (*R*)-Enantiomer	Tailing factor
Proposed method*	16.41	2.0	1.1
Column temperature 30°C	18.60	1.6	1.2
Column temperature 40°C	14.52	1.8	1.3
Flow rate (0.9 mL/min)	18.22	1.7	1.3
Flow rate (1.1 mL/min)	14.78	1.6	1.2

Robustness

The robustness of a method is the ability of the method to remain unaffected by small changes inparameters such as flow rate and column temperature. The chromatographic resolution between rivaroxaban and its (R)-enantiomer was used to evaluate the robustness of the method. The resolution between Rivaroxaban and its (R)-enantiomer was more than 1.5 under all tested separation conditions (Table 4) demonstrating sufficient robustness.

CONCLUSION

A simple, rapid, and accurate enantio selective High Performance Liquid Chromatography (HPLC) method was successfully developed for separating the enantiomer from rivaroxaban. Method validation was carried out using a Chiralcel OD-H column due to the better chromatographic results achieved on the column. The validated method was demonstrated to be accurate, precise, selective, sensitive and robust. The developed and validated method can be implemented for the chiral purity testing of Rivaroxaban and also for the quantitative determination of chiral impurities in bulk materials.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Xarelto. Summary of Product Characteristics". Bayer Schering Pharma AG; 2008.

2. http://www.rxlist.com/xarelto-drug.htm
3. Roehrig S, Straub A, Pohlmann J, Lampe T, Pernerstorfer J. Discovery of thenovel 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 factorXa inhibitor. J Med Chem 2005;48:5900-8.
4. European Medicines agency. CHP Assessment Report for Xarelto; 2008.
5. Weinz C, Schwarz T, Kubitza D, Mueck W, Lang D. Metabolism and excretion of rivaroxaban, an oral, direct factor Xa inhibitor, in rats, dogs, and humans. Drug Metab Disposition 2009;37:1056-64.
6. Mustafa Celebier, Tuba Recber, Engin Kocak, Sacide Altınoz. RP-HPLC method development and validation for estimation of rivaroxaban in pharmaceutical dosage forms. BJPS 2013;49:2.
7. Lories IB, Mostafa AA, Girges MA. High performance liquid chromatography, TLC Densitometry, First-derivative and First-derivative ratio spectrophotometry fordetermination of rivaroxaban and its alkaline degradates in bulk powder and its tablets. J Chromatogr Sep Tech 2013;4-9.
8. Chandra Bala Sekaran, Vankayalapati Hima Bind, Mittapalli Rupa Damayanthi, Anaparthi Sireesha. Development and validation of UV spectrophotometric method for the determination of rivaroxaban. Der Pharm Chem 2013;5(4):1-5.
9. ICH Harmonized Tripartie Guidline Q2A Text on validation of analytical procedures; 2005.