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Designing and Quantification of impurity profile in an Anticoagulant drug substance Rivaroxaban

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Abstract

Non vitamin K antagonists are direct factor Xa inhibitor. Factor Xa is a protein that helps blood clots to form, Rivaroxaban is such an example being used to treat disease like deep vein thrombosis, pulmonary emboli, and atrial fibrillation. For its long duration treatment,to reduce its side effects and enhance efficacy strict quality measure is required.The objective of this study is to design impurity profile of Rivaroxaban scientifically, to set limit as per ICH Q3 and develop the accurate HPLC method.

Keywords: Rivaroxaban, Impurity profiling, HPLC method.

1. Introduction:

Rivaroxabanis5-chloro-N-({(5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl) phenyl]-1,3-oxazolidin-5yl}methyl)thiophene-2-carbinoxamide (Figure 1).

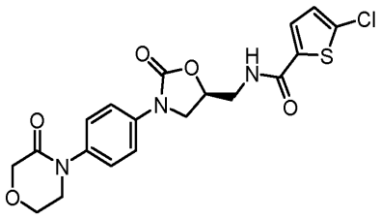

Figure 1: Rivaroxaban

Molecular mass 435.88

Molecular formula : $C_{19}H_{18}ClN_3O_5S$

Rivaroxaban is an oxazolidinone derivative anticoagulant that competitive reversible antagonist of activated factor X (Xa). Factor Xa is the active component of the prothrombinase complex that catalyses conversion of prothrombin (factor II) to thrombin (factor IIa). It is a highly selective direct Factor Xa inhibitor with oral bioavailability and rapid onset of action. Rivaroxabin does not inhibit thrombin (activated Factor II), and no effect on platelets have been demonstrated In

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November 2008 the Therapeutic Goods Administration approved new oral anticoagulant drug Rivaroxaban for the prevention of venous thrombosis in patients having elective knee or hip replacement.[1-2]

There is no official monograph available for Rivaroxaban or drugproduct in any pharmacopiea. A preliminary survey of literature for suitable method development for Rivaroxaban has been made.[3-4]Review of literature suggest that no extensive work has been carried out for the routine analysis of Rivaroxaban,[5-7]Which can address all process impurities and degradation profiles.Monitoring of impurity profiling is very important for quality of drug and patient safety purpose. Also literature survey shows few analytical methods were published for the estimation of Rivaroxaban during formulation and bio availability study for the assay purpose. But rare discussion is available for Rivaroxaban impurity profilestudy. This study shows detail discussion on monitoring of commercial rout of synthesis(Figure 2) and impurity profiling (Figure 3).Hencethe aim of the present work was to develop accurate and robust routine HPLC method

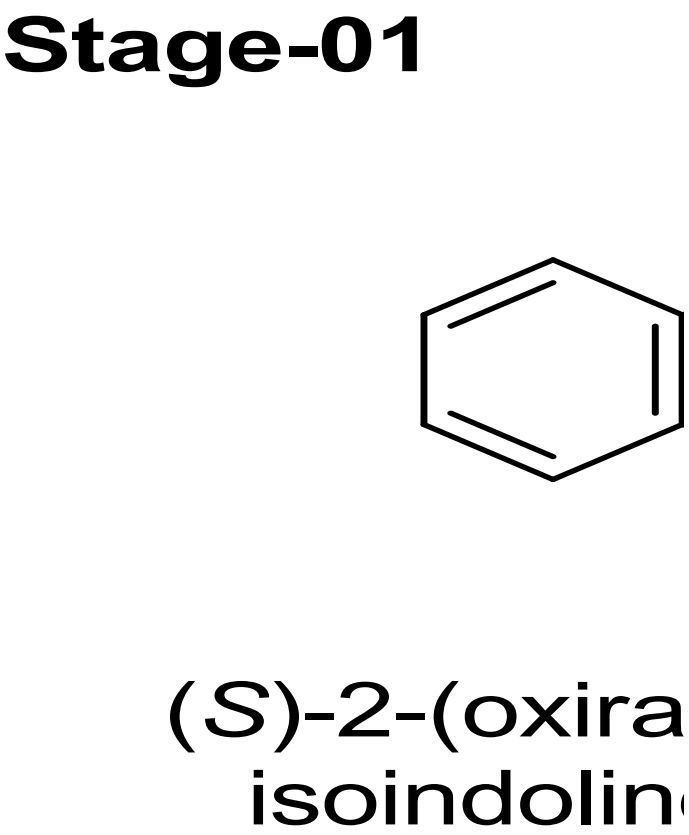

Figure 2: Commercial rout of synthesis for rivaroxaban

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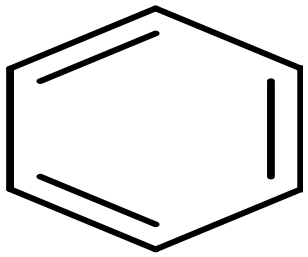

(S)-2-(oxira
isoindolin

Figure 3: Impurity profiling of rivaroxaban

2. Experimental

2.1. Material and reagents

Pure Rivaroxaban was obtained using commercial route of synthesis as per the process described in the Figure-2[9]. The related impurities including process impurities and degradant impurities(as described in Figure-3) were synthesised in house. The rivaroxaban standard and impurities were characterized using proton nuclear magnetic resonance and mass spectrometry equipped with HPLC. HPLC grade acetonitrile,methanolwere procured from J T Baker. Analytical grade potassium dihydrogen phosphate and orthophosphoric acid obtained from Merck chemicals. HPLC grade water obtained from Millipore system was used throughout the analysis.Ion pair reagent Octane sulfonic acid was purchased of Ranchem make

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3. RP-HPLC method:

3.1Optimization experiments:

In the process of developing RPHPLC method three key parameters were studied which influence the selectivity such as chemistry of stationery phase, pH of the buffer, and organic modifiers. Phophate buffer with Octane sulphonic acid,pH was screened as pH3.0,4.0 and 5.0. HPLC columns were used for development of method were Inertsil ODS 3V, Zorbax phenyl, Kromasil C18 and ZorbaxSB C18. Methanol and Acetonitrile were chosen individual and in different ratio as organic modifier. The impurities spiked solution in Rivaroxaban and sample of all stressed condition were studied and recorded and method was optimized with satisfactory resolutions among all impurities on ZorbaxSB C18 column.

3.2. Instrumentation and Chromatographic Conditions

Agilent HPLC 1200 (Agilent Technologies, Germany) equipped with photodiode array detector was used for method development, forced degradation studies and method validation. Zorbax SB C18 (250mmX4.6 mm 3.5 µ) HPLC column. Column thermostat at 45˚C was used for the impurities separation. Buffer was prepared using 0.02M of anhydrous potassium dihydrogen phosphate and 1 gm of Octane sulphonicacid solution was adjusted to pH 3.0 with orthophosphoric acid.Solvent mixture was prepared Acetonitrile:Methanol in ratio 820:180v/v.Mobile phase A was prepared by mixing Buffer and solvent mixture in ratio800 :200 v/v. Mobile phase-B was prepared by mixing Buffer and solvent mixture in ratio of 200:800 v/v. The flow rate and injection volumes were 1.0ml/min and 10µlrespectively. The analysis was carried out under the gradient condition as time$_{(min)}$/A(v/v):B (v/v); $T_{0.01}$/85:15, $T_{22.0}$/75:25, $T_{35.0}$/55:45, $T_{50.0}$/40:60 ,$T_{65.0}$/40:60, $T_{66.0}$/85:15 and $T_{75.0}$/85:15. The data was acquired at 240nm forImpurity 1, to Impurity 10; Run time kept 75 min. Chromatographic data processed by using chemstation and chromline HPLC soft ware. The photodiode array detector was used todetermine the peak purity of stressed sample.

3.3. Preparation of Solutions and Analytical Procedure

Diluent was prepared by mixing solution A and water in the ratio of 500 :500 v/v.Solution A was prepared by mixing methanol and Acetonitile in the ratio of 500 :500 v/v. The test sample solution having concentration of 1000 µg/mlwas prepared for the determination of related substances. The stock solution was prepared by dissolving each impurity (Impurity1, Impurity 2,Impurity3,Impurity4,Impurity5,Impurity6,Impurity7,Impurity8,Impurity9 and Impurity10) at concentration about 15 µg/mLin diluent and further diluted upto 1.5µg/ml along with Rivaroxaban standard at 1000 µg/ml to prepare the system suitable solution. The blank, system suitability solution and sample solution of 1000µg/ml, were injected separately and chromatographed under the optimized chromatographic conditions. The resolution NLT 2.0, between Impurity 8peak and Impurity 9 were set as system suitability criteria. All impurities were quantified against 0.1% rivaroxaban diluted standard solution applying the derived relative response factor(RRF). The relative retention time with respect to rivaroxaban peak and RRF of all impurities are as shown in Table-I.

Table-I: Rivaroxaban and its impurities elution order and relative response factor

Sr.No	Name	RRT	RRF
1	Impurity-1	0.10	0.70
2	Impurity-2	0.13	0.74
3	Impurity-3	0.26	1.00
4	Impurity-4	0.39	1.06
5	Impurity-5	0.45	1.53
6	Impurity-6	0.48	0.66
7	Impurity-7	0.58	0.57
8	Impurity-8	0.72	1.25
9	Impurity-9	0.77	1.05
10	Rivaroxaban	1.00	1.0
11	Impurity-10	1.16	0.57

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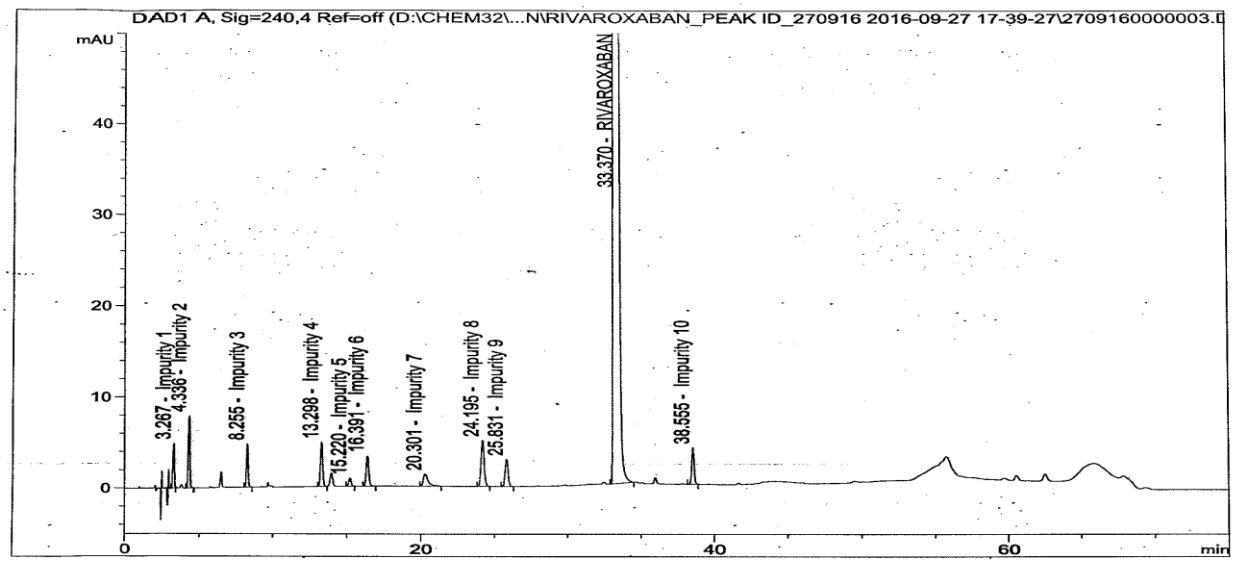

Figure 4:All impurities spiked at 0.15%level in Rivaroxaban

4. Validation

4.1. Specificity (Selectivity)

Specificity is the ability of method to measure the analyte in presence of its potential impurities. Stress testing of the drug substance performed to identify likely degradation impurities, which intern help to establish the degradation pathways and intrinsic stability of the molecule and validate the stability-indicating power of the analytical procedures used.[8-9]

The specificity of developed RP-HPLC method for Rivaroxaban was determined in presence of its impurities (Impurity 1 to Impurity10) and degradation products. Forced degradation studies were also performed on Rivaroxaban to provide an indication of the stability-indicating property and specificity of the proposed method. The dry degradation study was performed by exposing the sample to different stress conditions such as light (1.2 million lux hours), heat (80˚C for 12hours),hydrolytic condition (45°C, 75%RH for 48 Hrs.). Wet degradation was performed as acid hydrolysis (1 M HCl for 4 hrs at 80°C), base hydrolysis (1 M NaOH for 4 hrs at 80°C) and oxidation (5%v/v H_2O_2 for 4 hrs at 40°C)

The peak purity of all impurities and main peak was calculated and found complying. Mass balance was observed during degradation for all the stressed samples.

4.2. Linearity

Linearity solutions were prepared by quantitative dilutions of the stock solution of impurity standard and main drug standard to obtain solutions at LOQ to 250% of the specification limiti.e. known impurity at 0.15% level and unknown impuurity at 0.1% level.A series of solutions were prepared by quantitative dilutions of the stock solution of main drug to obtain solutions at 80% to 120% of the sample concentration.
Each solution was injected and areas were recordedThe linearity of peak areas versus different concentrations was evaluated for Rivaroxaban and its related impurities. The linear regression data for all the impurities ploted and correlation coefficient for all impurities was above 0.99.

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4.3. Limits of Detection and Quantification (LOD and LOQ)

According to ICH Q2 (R1) recommendations the limits of detection (LOD) and the limit of quantification (LOQ) for Rivaroxaban and its process related impurities (Impurity 1 to Impurity 10) were estimated by calibration curve method [standard deviation of the response (σ) and the slope (S)], by injecting the series of dilute solutions of known concentration. The values of LOD and LOQ found are as depicted in Table-2

Table-2: LOQ and LOD values for Rivaroxaban and its impurity

Sr.No	Name	LOQ	LOD	Sr.No	Name	LOQ	LOD
1	Impurity-1	0.033	0.019	7	Impurity-7	0.048	0.021
2	Impurity-2	0.025	0.012	8	Impurity-8	0.027	0.009
3	Impurity-3	0.026	0.011	9	Impurity-9	0.034	0.015
4	Impurity-4	0.021	0.006	10	Rivaroxaban	0.030	0.018
5	Impurity-5	0.037	0.015	11	Impurity-10	0.037	0.022
6	Impurity-6	0.043	0.012				

Precision was studied at the LOQ levelby injecting six in-dividual preparations of Rivaroxaban and its impurities, followed by the calculation of %RSD of the peaks areas. The %RSD of LOQ precision was below 10%.

4.4. Precision

The precision of method is degree of agreement between the results. Precision of the method was studied for system precision, method precision and intermediate precision. A standard solution of Rivaroxaban at 0.1% was injected for six time to determine the system precision of the method and %RSD was calculated for Rivaroxaban. The %RSD of system precision was found about0.69%.

Six separate test sample solutions of Rivaroxaban were prepared by spiking the related impurities (Impurity 1 to Impurity 10) at limit level(i.e.0.15% for known and 0.1% for unknown). The %RSD (n = 6) for each related impurities was evaluated and found in between 0.72% to 2.44%.The similar procedure of method precision was carried out by a different analyst, using different mobile phase and diluent preparations and instrument on a different day with different lot of same brand column for intermediate precision study. The %RSD of results for intermediate precision study was calculated and compared with the method precision results.

4.5. Accuracy (Recovery)

Accuracy of the method for all the impurities was determined by analyzingRivaroxaban sample solutions spiked with all the impurities at four different concentration levels of LOQ, 50 %,100 % and 250% of each at the specified limit in both methods. The recovery of all these impurities were found to be in-between the predefined acceptance criterion of 80.0% - 120.0% .

4.6. Stability of Analytical Solution

Rivaroxaban spiked with all impurities at specified level were prepared and analyzed immediately and after different time intervals up to 24 hrs to determine the stability of sample solution in both methods. The sample cooler temperature was maintained at about 25 °C and at about refrigerator temperature (2 –8°C). The results from these studies indicated that the sample solution was stable at room temperature and at 2 -8°C.

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4.7. Robustness

The chromatographic conditions were deliberately altered to evaluate the robustness of developed method. The resolution between closely eluting peakswas evaluated on altered chromatographic condition. To study the effect of flow rate on the resolution, the flow rate of mobile phase was altered by ± 0.1 mL/min (0.9 to 1.1 mL/min from 1.0 mL/min). The effect of column temperature on resolution was studied at 40°C and 50°C instead of 45°C. whereas all other mobile phase components were held constant similarly to study the pH effect,pH of buffer was altered by ± 0.2keeping rest parameters same. Buffer :solvent mixture was studied by changing composition ±2% absolute of solvent mixture. All these parameters were studied by changing one parameter only at a time. The resolution between all known and unknown peaks present in sample was greater than 1.5 in all the deliberate varied chromatographic conditions indicating the robustness of the method.

5. Conclusion

The proposed RP-HPLC method was simple, specific, linear, sensitive, accurate, precise, and robust for separation and quantification of Rivaroxaban and its process related, degradation related impurities. Since there is no official monograph of rivaroxaban and drug product in any pharmacopeia; hence this method can be use for future analysis purpose.

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