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Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Rivaroxaban and Clopidogrel Bisulphate in Pharmaceutical Dosage Form

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Received: 10 December 2019 Accepted: 1 January 2020 Published: 15 January 2020

Abstract

A simple, specific, accurate and stability-indicating reversed phase high performance liquid chromatographic method was developed for the simultaneous determination of Rivaroxaban and Clopidogrel, using a C18 (25cm x 0.46 cm) Hypersil BDS column and a mobile phase composed of buffer (pH 4.5): methanol (70:30). The detection was carried out at wavelength 214 nm. The retention times of Rivaroxaban and Clopidogrel were found to be 3.300 min and 4.740min, respectively. Linearity was established for Rivaroxaban and Clopidogrel in the range of 2-6?g/ml and 7.5-22.5?g/ml, respectively. The percentage recoveries of Rivaroxaban and Clopidogrel were found to be 100.09

Index terms— clopidogrel bisulphate, rivaroxaban, rp-hplc, stability indicating method.

1 Introduction

Ivaroxaban ((S)-5-chloro-N-{[2-oxo-3-[4-(3-oxomorpholin-4-yl) phenyl] oxazolidin-5-yl] methyl} thiophene-2-carboxamide) (fig. 1) is an anticoagulant and the first orally active direct factor Xa inhibitor [1] . Clopidogrel Bisulphate methyl (2S)-2-(2chlorophenyl)-2-{4H, 5H, 6H, 7H-thieno [3, 2-c] pyridin-5-yl} acetate (fig. **??**)is an antiplatelet agent structurally and pharmacologically similar to ticlopidine. It is used to inhibit blood clots in a variety of conditions such as peripheral vascular disease **??**2] . Fixed dose combination (FDC) for the probing drugs is not available commercially, yet this FDC has not been listed in any of the common pharmacopoeia.

When go through the literature lead to the occurrence of Author ?: Department of Quality Assurance, Ali-Allana College of Pharmacy, Akkalkuwa 425415, Dist. Nandurbar, Maharashtra., India. e-mail: naimmajan@gmail.com Author ? ?: Department of Quality Assurance, Ali-Allana College of Pharmacy, Akkalkuwa, 425451, Nandurbar, Maharashtra, India.

various singular methods for this drug like, for Rivaroxaban RP-HPLC [3][4][5][6][7] , RP-HPLC and UPLC (Forced Degradation study) [8] , RP-UPLC (Stability indicating Method) [9] , RP-HPLC (Stability indicating Method) [10] , RP-HPLC (Stability indicating Dissolution Method) [11] , RP-HPLC and TLC [12] , HPTLC [13] , UV Spectrophotometry [14] and for Clopidogrel Bisulphate Chiral Chromatography **??**15][16] , RP-HPLC [17][18][19][20] , Stability Indicating RP-HPLC [21][22] . In combination of this drug only RP-HPLC method were available in mobile phase and also in plasma and urine **??**23-24-25] . However no stability indicating RP-HPLC method was set up for this combination.

We are involved newly to conduct investigation relating to stability indicating RP-HPLC method development of Rivaroxaban and Clopidogrel for fill this information gap. It was tried to develop and validate RP-HPLC method with stability indicating properties for this combination (Rivaroxaban and Clopidogrel Bisulphate). We expect the inclusion of this knowledge in the current literature will be benefit for the pharmaceutical industries for support the quality of their products holding these active ingredients and also the execution agencies in broad to evaluate the quality of the marketed preparations.

2 II.

3 Experimental

Chemical and reagent: Pure Clopidogrel (CLP) and Rivaroxaban (RIV) were obtained as a gift sample from Remus Remedies. As Sample Clopidogrel 75mg and Rivaroxaban 20mg Synthetic Mixture is used. HPLC grade Methanol, Potassium dihydrogen, Ammonium Acetate, HPLC Grade High purity deionized water were obtained from Merck specialties Pvt Ltd., Mumbai.

4 Instrumentation and materials:

The liquidchromatographic system was of Thermo separation Product TSP UV 2000, which consisted a gradient pump, variable wavelength, programmable UV/Vis detector, a manual injection facility with 20 ?l fixed loop. The chromatographic analysis was performed using spinchrom software on a C18 Hypersil BDS column (25cm x 0.46 cm with 5?m particle size). In addition, an electronic balance (CP-124S Sartorius, Germany), a pH meter (Electroquip's Digital pH meter)were used in this study.

5 Chromatographic conditions:

The elution of CLP and RIV was obtained by running HPLC in isocratic mode using Phosphate Buffer (pH 4.5): Methanol (70:30).Flow rate was maintained at 1.0 ml/min with run time of 6 min. The retention time for RIV was obtained 3.300 minand CLP was obtained 4.740min. Detection was performed at 214 nm. Mobile phase was previously filtered through Whatman filter paper no 41. a) Preparation of standard solutions CLP standard stock solution (150?g/ml): A 15 mg of CLP was weighed and transferred to a 100 ml volumetric flask. Volume was made up to the mark with mobile phase. RIVstandard stock solution(40?g/ml): A 40 mg of RIV was weighed and transferred to a 100 ml volumetric flask. Volume was made up to the mark with mobile phase, taken 10ml from this solution and transferred to 100ml volumetric flask and volume was made up with methanol.

Preparation of standard solution of binary mixtures of CLP (15 ?g/ml) and RIV(4 ?g/ml): Take 1 ml from the CLP stock solution and 1ml from RIV stock solution and transferred to 10 ml volumetric flask and volume made up to the mark by mobile phase which was used in particular trials.

6 b) Preparation of formulation solution

Sample Stock Solution (CLP 150 ?g/ml, RIV 40 ?g/ml): Take Tablet powder equivalent to 15 mg of CLP and 4 mg of RIV was transferred to a 100 ml volumetric flask, Add 60 ml Mobile phase and Shake for 15 min and make up volume with Mobile phase. The solution was filtered through Whatman filter paper no. 42.

Working Sample Preparation (CLP 15 ?g/ml, and RIV 4 ?g/ml): Take 1 ml from standard stock solution and transferred to 10ml volumetric flask and made up volume up to the mark with the mobile phase. c) Forced degradation study [26] Acid degradation: Acid decomposition studies were performed by transferring 1 ml of stock solution in to 10 ml of volumetric flask. 2ml of 0.1 N Hydrochloride solutions was added and mixed well and put for 5 hrs. at room temperature. Then the volume was adjusted with diluent to get 15?g/ml for CLP and 4?g/ml for RIV.

Base degradation: Basic decomposition studies were performed by transferring 1ml of stock solution in to 10ml of volumetric flask. 2 ml of 0.1 N NaOH solutions was added and mixed well and put for 3 hrs at room temperature. Then the volume was adjusted with diluent to get 15?g/ml for CLP and 4?g/ml for RIV.

Oxidative degradation: Oxidative decomposition studies were performed by transferring 1ml of stock solution in to 10 ml of volumetric flask. 2 ml of 3% H 2 O 2 solutions was added and mixed well and put for 6 hrs at room temperature. Then the volume was adjusted with diluent to get 15?g/ml for CLP and 4?g/ml for RIV.

Photo degradation: Photo degradation studies were performed by transferring 1ml of stock solution in to 10 ml of volumetric flask. The volumetric flask was kept in UV Chamber for 12 hrs. Then the volume was adjusted with diluent to get 15?g/ml for CLP and 4?g/ml for RIV.

Thermal degradation: Thermal degradation studies were performed by transferring 1ml of stock solution in to 10 ml of volumetric flask. The volumetric flask was stored in oven at 80°C for 5 hrs. Then the volume was adjusted with diluent to get 15?g/ml for CLP and 4?g/ml for RIV.

Method validation: After method development, the method was validated in compliance with ICH guidelines. The method was validated for Accuracy, Precision, Reproducibility, Specificity, Limit of Detection, Limit of Quantitation, Linearity and Range, Ruggedness and Robustness. System suitability is an integral part of chromatographic method. These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. System suitability tests are based on the concept that the equipment, electronics, analytical operations and samples constitute an integral system that can be evaluated as a whole. System suitability testing provides assurance that the method will provide accurate and precise data for its intended use. Observed values for system suitability is show in Table 1. The linearity for RIV and CLP were assessed by analysis of combined standard solution in range of 2-6 ?g/ml and 7.5-22.5 ?g/ml respectively, Correlation coefficient for calibration curve RIV and CLP was found tobe0.998 and 0.999 respectively The regression line equation for RIV and CLP are as following:

For RIVy = 55.905x + 0.2138 and for CLP y = 16.145x -0.4796

7 e) Precision

The precision of the method was demonstrated by repeatability study, inter-day precision and intra-day precision. In the repeatability study, six replicates of the same concentration of working standard solutions were prepared and injected and chromatograms were recorded. The results obtained were shown in Table 2. In inter-day precision and intra-day precision, three replicates of three different concentration of working standard solution were prepared and injected and chromatograms were recorded. The results obtained were shown in Table 3. Accuracy of the method was confirmed by recovery study from formulation at three level of standard addition.2 µg/ml drug solution for RIV and 7.5µg/ml drug solution for CLP was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 214 nm. The amount of RIV and CLP was calculated at each level and % recoveries were computed. The results are shown in table **??**. Percentage recovery for Rivaroxaban was 0.718%-1.357 %, while for Clopidogrel it was found to be in range of 0.649 %-1.110 % Following parameters were changed one by one and their effect was observed on system suitability for standard preparation. (a)Flow rate of mobile phase was changed (± 0.2 ml/min) 0.8 ml/min and 1.2 ml/min. (b) Ratio of Mobile phase was changed (±2) Buffer: Methanol (72:28) and Buffer: Methanol (68:32). (c)pH of Buffer was changed (±0.2), pH 4.3 and pH 4.7.The effect of changes was found to be within the acceptance criteria as shown in table 6. The % RSD should be less than 2%. Triturate 20 tablets, take tablet powder equivalent to 15 mg of CLP and 4 mg of RIV was transferred to a 100ml volumetric flask, Add 60 ml Mobile phase and Shake for 15 min and make up volume with Mobile phase. Take 1 ml from this stock solution and transferred to 10 ml volumetric flask and made up volume up to the mark with the mobile phase. Inject above Solution 20 ?l for assay analysis. The solution was filtered through What man filter paper no. 42. The results are given in Table 7.

8 Conclusion

The Combined dosage form of Rivaroxaban and clopidogrel are not available commercially. But individually rivaroxaban is used as an anticoagulant and it is the first orally active direct factor Xa inhibitor and clopidogrel is used as an antiplatelet agent. Various methods are reported for the analysis of individual drug and in combination with other drugs but no stability indicating HPLC method reported for these two drugs in combined dosage form. Therefore, a novel RP-HPLC method has been developed for the simultaneous estimation of Rivaroxaban and Clopidogrelin combination. The optimized chromatogram was run for appropriate minutes with mobile phase Phosphate buffer (Ph 4.5): Methanol (70:30). Data related to peak like area, height, retention time, resolution etc. were recorded using software. Thermo scientific, C 18 (25cm×0.46cm) Hypersil BDS, Mobile Phase Phosphate buffer, pH 4.5: Methanol (70:30) with Flow Rate 1.0 ml/min and Runtime 6 min Injection volume of 20.0 ?l. The detection was carried out at wavelength 214 nm. It was found to be simple, precise and accurate. In this stability indicating RP-HPLC methods were developed by degradation of sample and compared with standard. The % RSD was also less than 2 % showing high degree of precision of the proposed method. The proposed method can be used for routine analysis of Rivaroxaban and Clopidogrel in combined dosage form. It can be also used in the quality control in bulk manufacturing. [1] [2]

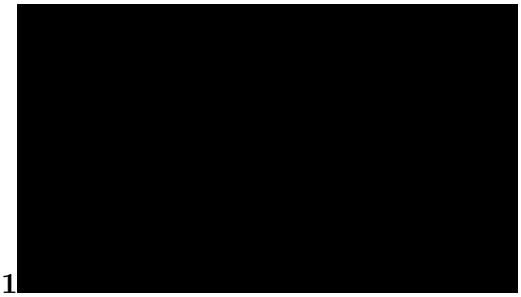

Figure 1: Figure 1 :

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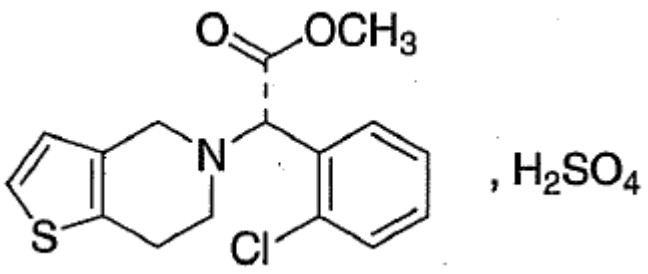
[2] © 2020 Global JournalsStability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Rivaroxaban and Clopidogrel Bisulphate in Pharmaceutical Dosage Form



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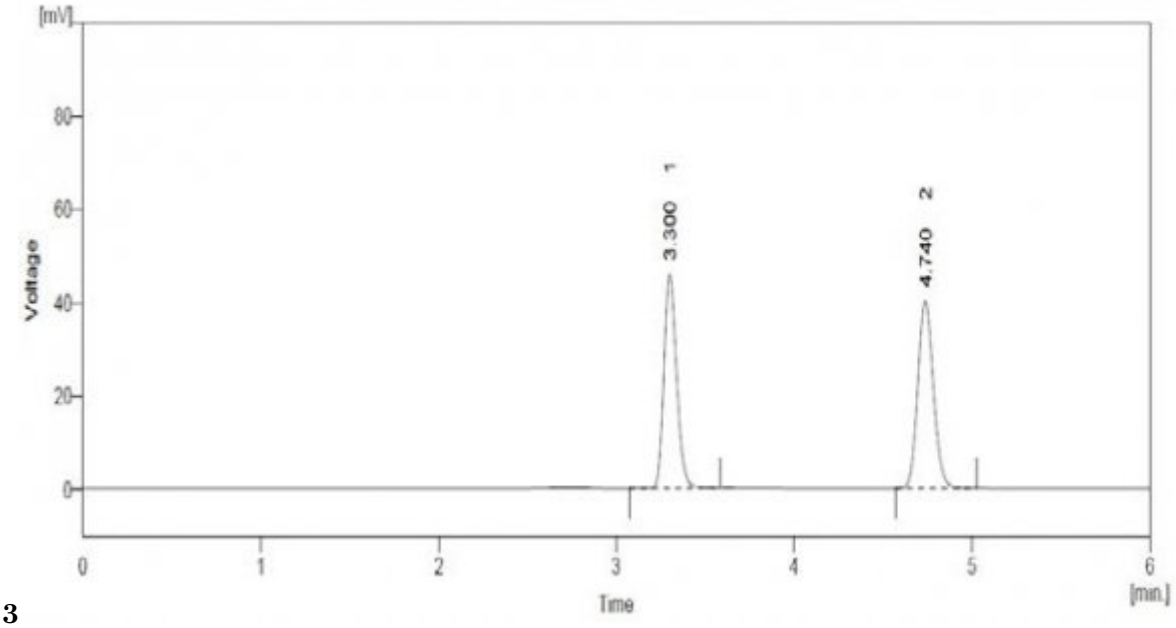

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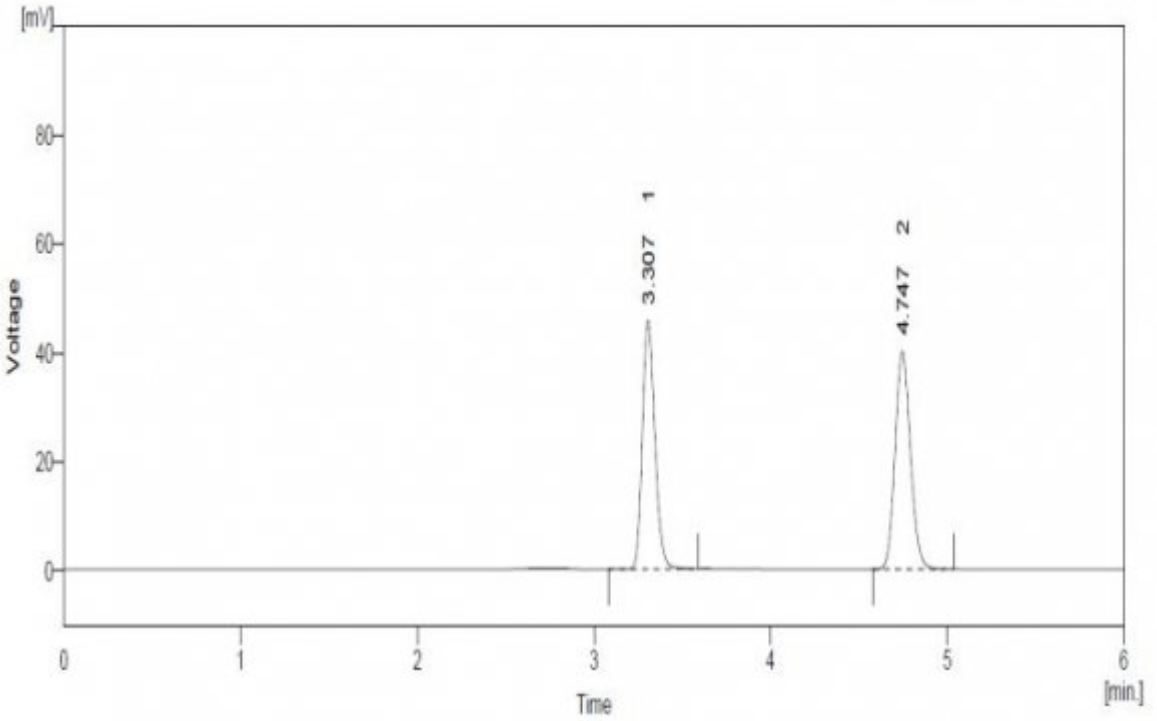
Figure 3: Figure 3 :



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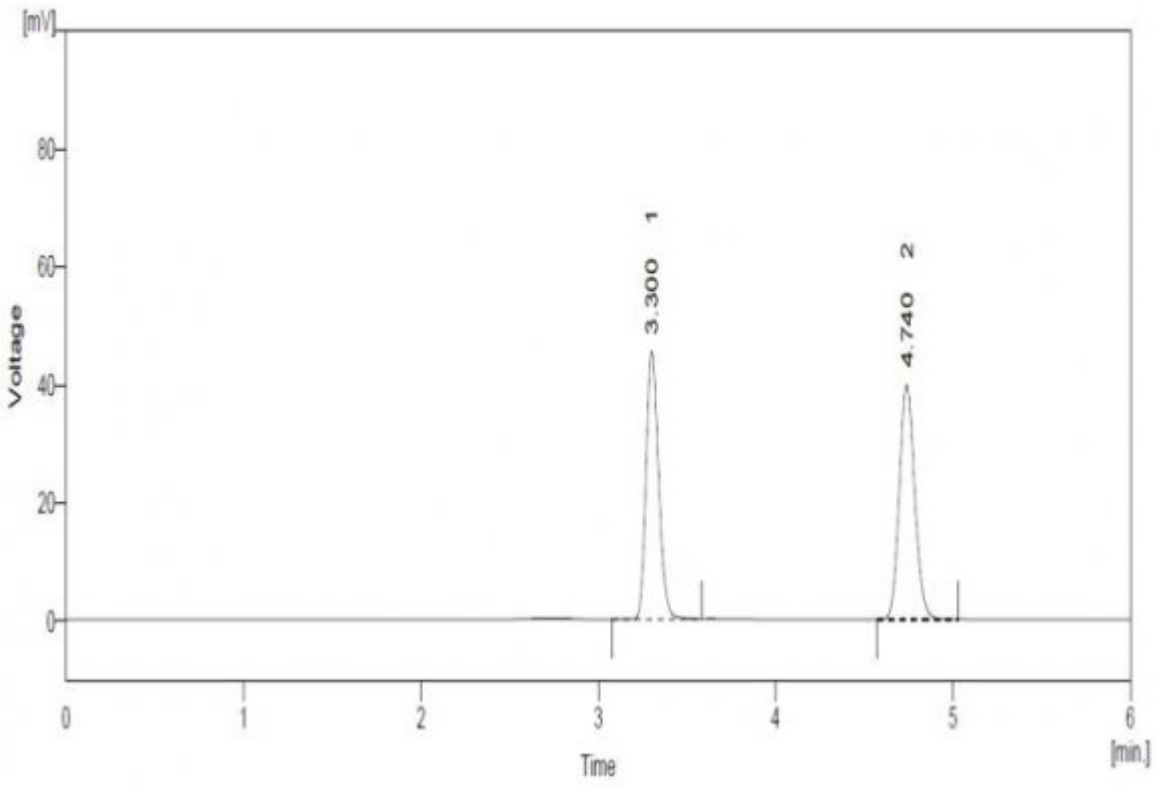

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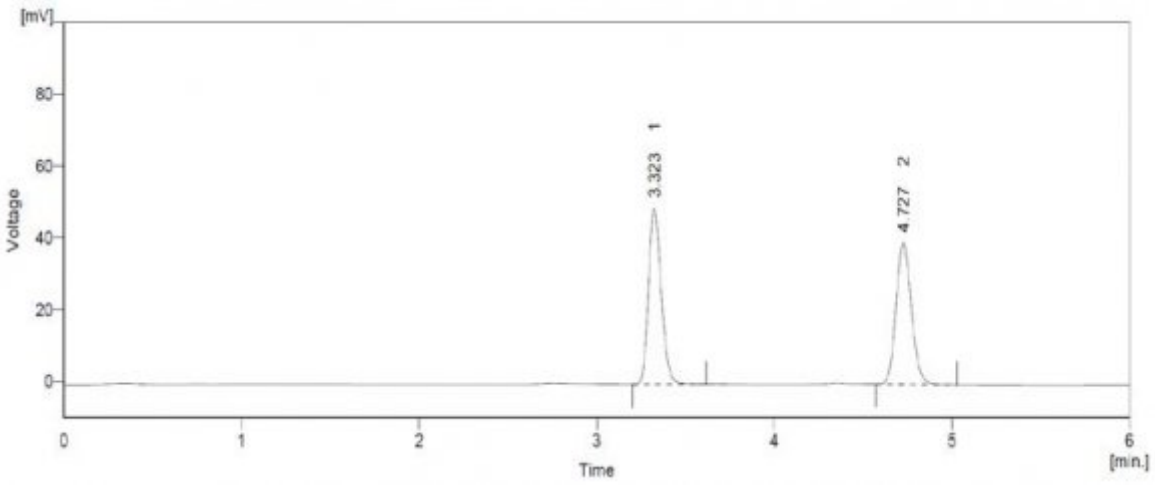
Figure 5: Figure 6 : 7 :



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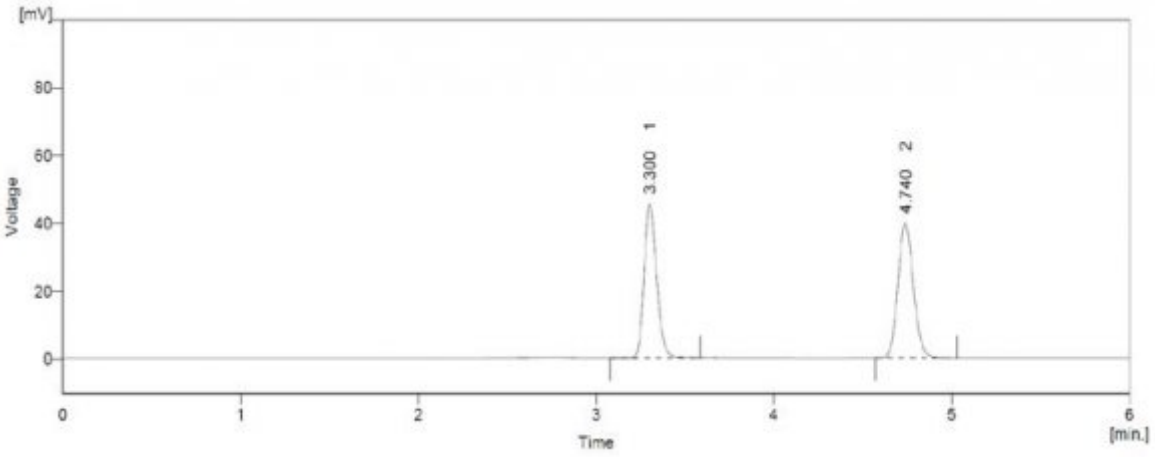

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Figure 7: Figure 8 :

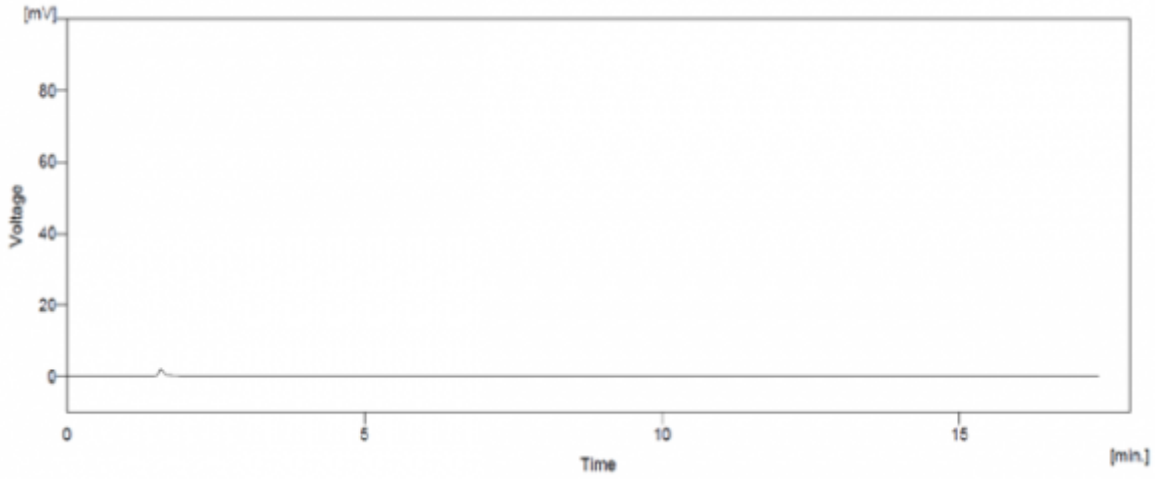

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Figure 8: Figure 9 :Figure 10 :Figure 11 :

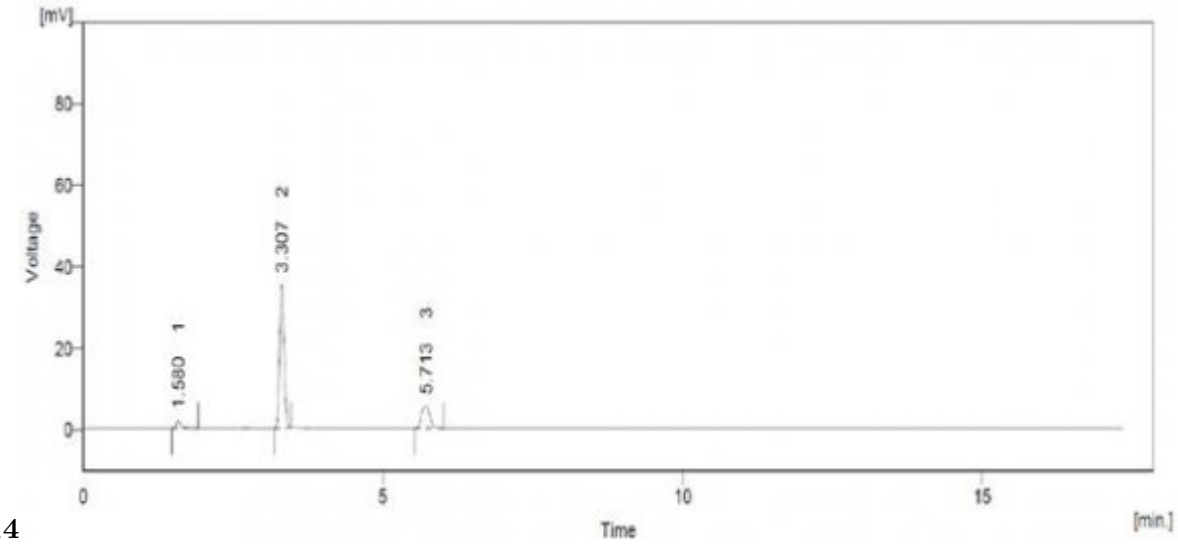

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Figure 9: Figure 12 :Figure 13 :Figure 14 :

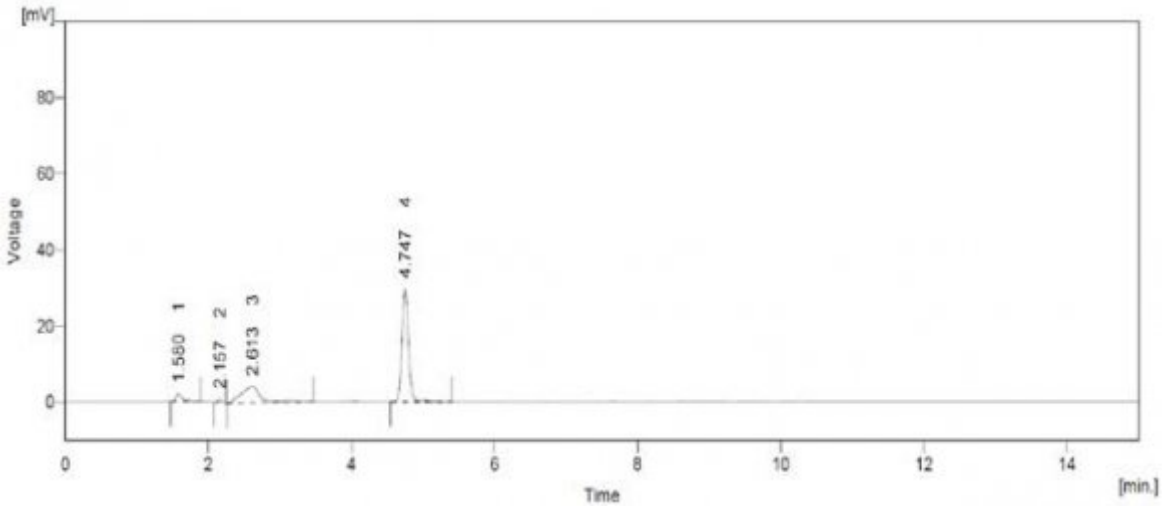

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Figure 10: Figure 16 :Figure 17 :Figure 18 :Figure 19 :

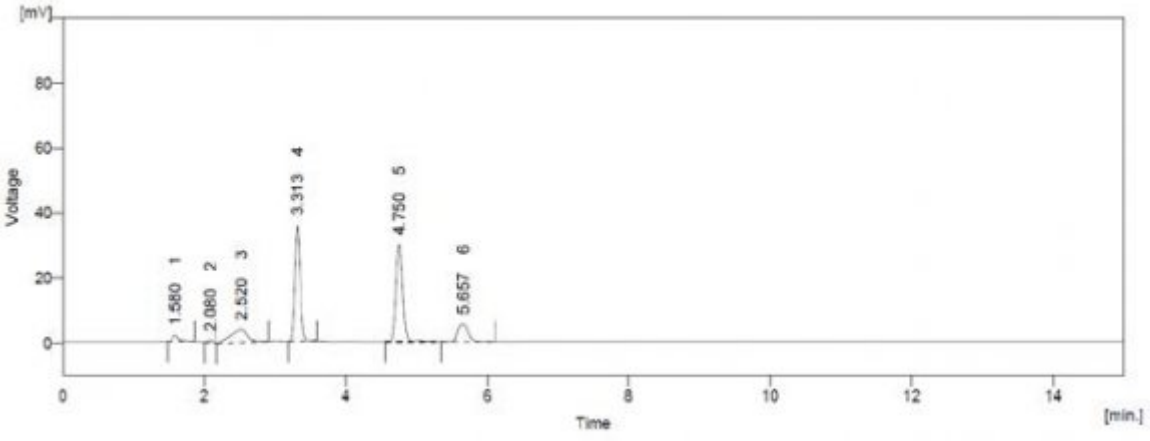

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Figure 11: Figure 20 :Figure 21 :Figure 22 :Figure 23 :

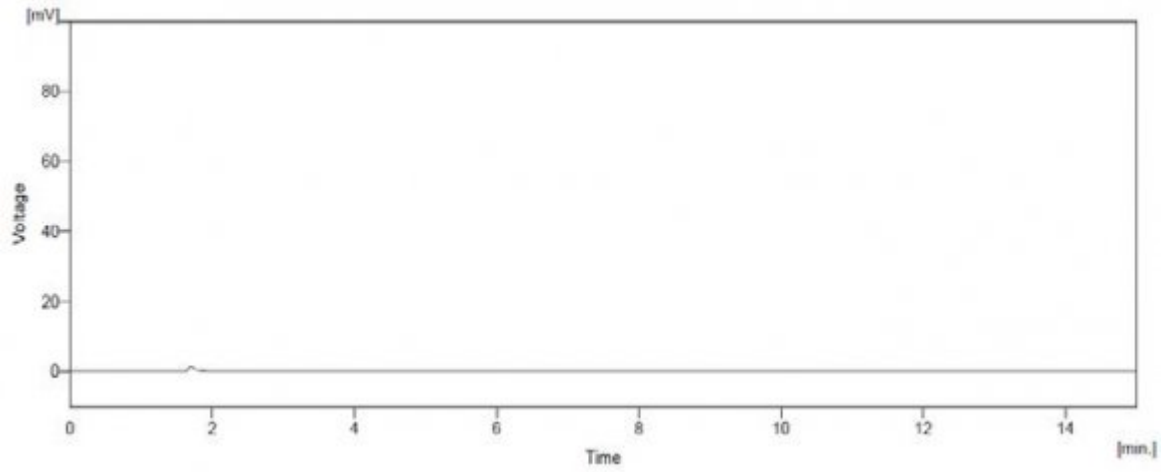

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Figure 12: Figure 24 :Figure 25 :Figure 26 :Table 9 :

1

Parameters	RIV	CLP
Retention Time	3.300	4.740
Theoretical Plates	9427	13320
Asymmetry	1.316	1.160
Resolution	9.593	

[Note: c) Validation of RP -HPLC method i. Specificity The Chromatograms of Clopidogrel and Rivaroxaban standards and Clopidogrel and Rivaroxaban sample show no interference with the Chromatogram of Clopidogrel and Rivaroxaban Blank, so the Developed method is Specific.]

Figure 13: Table 1 :

2

Drugs	Mean Area (n=6)	S.D	%R.S.D
RIV	224.773	1.058	0.471
CLP	242.442	1.451	0.599
n-Number of estimations			

Figure 14: Table 2 :

3

Parameter	Drug	Amount Taken (?g mL-1)	Mean Area Found (n=3)	S.D	%R.S.D
		2	113.066	0.556	0.451
	RIV	4	225.837	1.696	0.751
Intraday precision		6 7.5	335.157 122.28	1.21 0.754	0.368 0.616
	CLP	15	243.516	1.319	0.541
		22.5	362.858	1.696	0.467
		2	112.632	0.972	0.863
	RIV	4	224.325	1.237	0.552
Interday precision		6 7.5	333.162 122.769	3.512 0.388	1.054 0.316
	CLP	15	241.954	1.786	0.738
		22.5	360.46	4.525	1.255

n-Number of estimations
Accuracy

Figure 15: Table 3 :

5

LOD

Figure 16: Table 5 :

6

DrugConc. Level(%)	Sample amount added(µg/ml) (n=3)	Standard amount added(µg/ml) (n=3)	Mean of amount recovered (µg/ml) (n=3)	% Mean recovery	S.D	% R.S.D
80	2	1.6	1.601	100.081	1.358	1.357
RIV 100	2	2	1.992	99.594	0.716	0.718
120	2	2.4	2.415	100.623	0.734	0.729
80	7.5	6	6.001	100.028	0.649	0.649
CLP 100	7.5	7.5	7.474	99.656	0.948	0.951
120	7.5	9	8.975	99.728	1.107	1.11

Figure 17: Table 6 :

7

h) Stability indicating method

Figure 18: Table 7 :

.1 Acknowledgement

The authors are thankful to president of JIIU's G. M. Vastanvi and Principal for their encouragement and support. We also wish to thanks to Mr. Ketan Patel, Molecule Laboratory and Remus Remedies Ahmedabad, Gujarat.

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