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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF RIVAROXABAN BY USING RP-HPLC METHOD

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ABSTRACT

A new, simple, precise, accurate and reproducible RP-HPLC method for estimation of Rivaroxaban in bulk and Pharmaceutical formulations. Separation of Rivaroxaban was successfully achieved by using column like Xterra C18(150 x 4.6mm, 5µm) or equivalent in an isocratic mode utilizing mobile phase was optimized to 0.1% Octasulphonic acid buffer (pH 3.0): Acetonitrile in the proportion of 40: 60 v/v at a flow rate of 1.0ml/min and eluate was monitored at 281nm with a retention time of 2.235min for Rivaroxaban. The method was validated and their response was found to be linear in the drug concentration range of 50µg/ml to150µg/ml for Rivaroxaban. The values of the correlation coefficient were found to 0.999 for Rivaroxaban. The LOD and LOQ for Rivaroxaban were found to be 2.219 and 2.221 respectively. This method was found to be good percentage recovery which indicates that the proposed method is highly accurate. This method was extensively validated according to ICH guidelines for accuracy, precision, linearity, robustness and system suitability.

INTRODUCTION

Rivaroxaban is 5-chloro n-{[(5S)-2-oxo-3-[4-(3-xomorpholin-4-yl) phenyl]-1, 3-oxozolidin-5-yl] methyl} thiophene-2-corboxamide, [1]. It belongs to the class of direct factor Xa inhibitor approved for the prevention of venous thromboembolic events in patients who have undergone total hip or total knee replacement surgery. RXN blocks the amplification of the intrinsic and extrinsic pathway of coagulation cascade by binding directly to the catalytic pocket of factor Xa and thereby preventing the formation of thrombus [2]. Literature survey revealed that studies had been carried out on Rivaroxaban on RP-HPLC, LCMS/MS, HPTLC [3-13]. The developed method was validated as per ICH guidelines [14].

Method Development

Preparation of buffer and mobile phase

Preparation of 0.1% Octasulphonic acid (buffer)

Accurately weighed 1 grams of Octasulphonic acid was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.0 with Orthophosphoric acid.

Preparation of mobile phase

Accurately measured 400 ml (40%) of above buffer and 600 ml of Acetonitrile HPLC (60%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

Preparation of the Rivaroxaban Standard & Sample Solution

Standard Solution Preparation

Accurately weigh and transfer 25mg of Rivaroxaban working standard into a 25ml clean dry volumetric flask add about 10ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.75 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. (75ppm of Rivaroxaban)

Sample Solution Preparation

Accurately weigh 5 tablets crush in mortor and pestle and transfer equivalent to 25mg Rivaroxaban (marketed formulation=132.5mg of tablet Powder) sample into a 25ml clean dry volumetric flask add about 10 ml of Diluent and sonicate it up to 30 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.44 micron Injection filter. (Stock solution)

Further pipette 0.75 ml of Rivaroxaban from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. (75ppm of Rivaroxaban)

Procedure

Inject 5 µL of the standard, sample into the chromatographic system and measure the areas for Rivaroxaban peaks and calculate the %Assay by using the formulae.

Method Validation

Precision

Procedure

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Sample Solution Preparation

Accurately weigh 5 tablets crush in mortor and pestle and transfer equivalent to 25mg Rivaroxaban (marketed formulation=132.5mg of tablet Powder) sample into a 25ml clean dry volumetric flask add about 10 ml of Diluent and sonicate it up to 30 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.44 micron Injection filter. (Stock solution) Further pipette 0.75 ml of Rivaroxaban from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. (75ppm of Rivaroxaban)

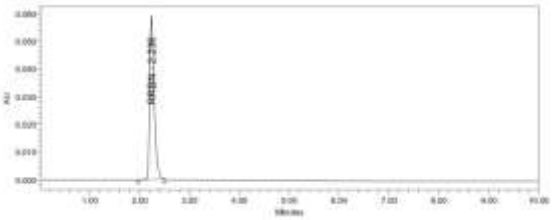

Fig. 1: Chromatogram for precision

Table 1(a): Results for Precision

Injection	Area for Rivaroxaban
Injection-1	347358
Injection-2	345898
Injection-3	349624
Injection-4	351347
Injection-5	345567
Injection-6	349045
Average	341839.8
Standard Deviation	2261.2
%RSD	0.6

Acceptance Criteria: The % RSD for the area of six standard injections results should not be more than 2%**.**

Table 1(b) : % Assay results for method precision

Sample Name	% Assay for Rivaroxaban
Method precision-1	100.28
Method precision-2	100.758
Method precision-3	100.27
Method precision-4	100.44
Method precision-5	100.03
Method precision-6	100.16
Average	100.26
Standard deviation	0.15
% RSD	0.15

Acceptance Criteria: The % RSD for the area of six standard injections results should not be more than 2%.

Specificity

For Specificity Blank and Standard are injected into system. There is no any interference of any peak in blank with the retention time of the analytical peaks.

Procedure: Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy

-150% solutions. Calculate the Amount found and Amount added for Rivaroxaban and calculate the individual recovery and mean recovery values.

Linearity

Preparation of Level – I (25 ppm of Rivaroxaban) 0.25 ml of above stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – II (50 ppm of Rivaroxaban) 0.5 ml of above stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – III (75 ppm of Rivaroxaban) 0.75 ml of above stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – IV (100 ppm of Rivaroxaban)

1.0 ml of above stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent

Preparation of Level – V (125ppm of Rivaroxaban)

1.25 ml of above stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent

Procedure

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Limit of Detection: (for Rivaroxaban)

Preparation of 0.26µg/ml solution

Accurately weigh 5 tablets crush in mortor and pestle and transfer equivalent to 25mg Rivaroxaban (marketed formulation=132.5mg of tablet Powder) sample into a 25ml clean dry volumetric flask add about 10 ml of Diluent and sonicate it up to 30 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.44 micron Injection filter. (Stock solution)

Further pipette 0.75 ml of Rivaroxaban from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. (30ppm of Rivaroxaban) Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents. Further pipette 0.34ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Limit of Quantification

Preparation of 0.83µg/ml solution:

Accurately weigh 5 tablets crush in mortor and pestle and transfer equivalent to 25mg Rivaroxaban (marketed formulation=132.5mg of tablet Powder) sample into a 25ml clean dry volumetric flask add about 10 ml of Diluent and sonicate it up to 30 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.44 micron Injection filter. (Stock solution) Further pipette 0.75 ml of Rivaroxaban from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. (30ppm of Rivaroxaban) Further pipette 1 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.Further pipette 1.1 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Robustness

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

A. The flow rate was varied at 0.9 ml/min to 1.1 ml/min.

Standard solution 75ppm of Rivaroxaban was prepared and analysed using the varied flow rates along with method flow rate. On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.

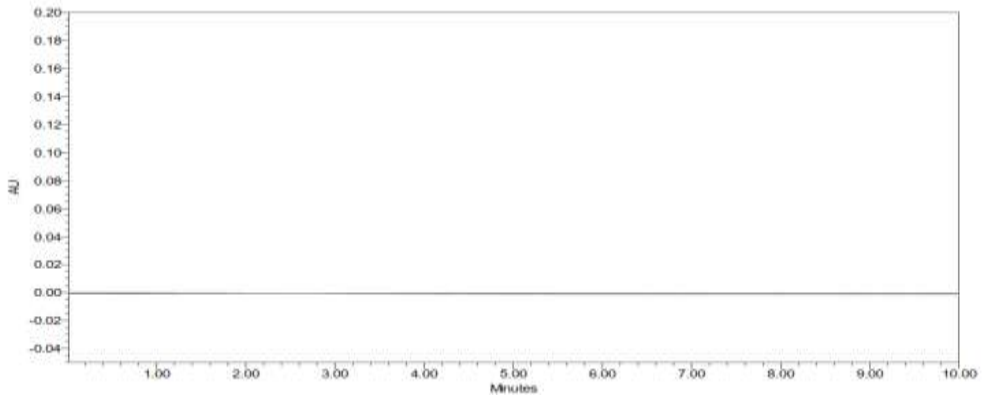

Fig. 2: Chromatogram for specificity

Table 2: Accuracy results for Rivaroxaban

%Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1745738	12.5	12.60	100.49	100.02
100%	347420	25	25.00	99.99	
150%-3	518990	37.5	37.34	99.58	

**n=3(mean area of three replicates)*

Acceptance Criteria

The % Recovery for each level should be between 98.0 to 102.0%

Table 3: Linearity Results: (for Rivaroxaban)

S. No	Linearity Level	Concentration	Area
1	I	25	117116
2	II	50	234231
3	III	75	351347
4	IV	100	458463
5	V	125	585578
Correlation Coefficient			0.999

Acceptance Criteria: Correlation coefficient should be not less than 0.99.

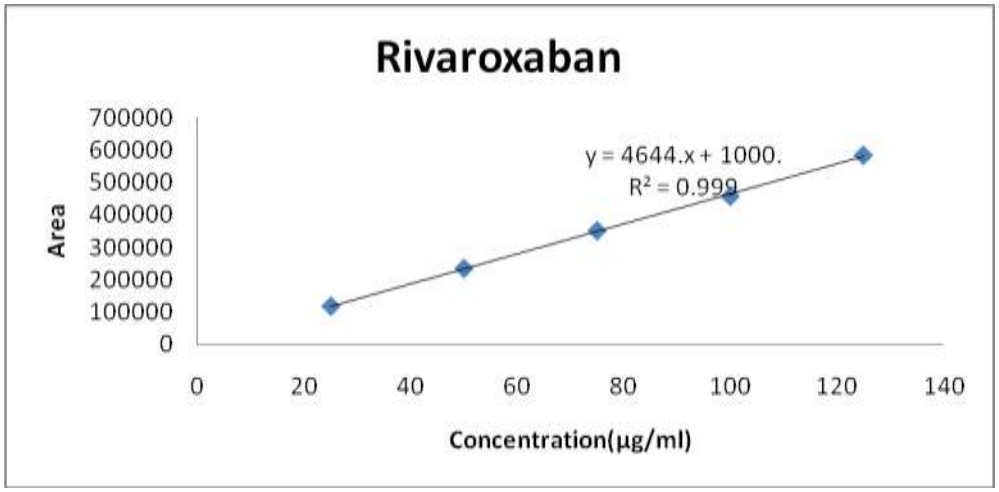

Fig. 3: Calibration Curve for linearity

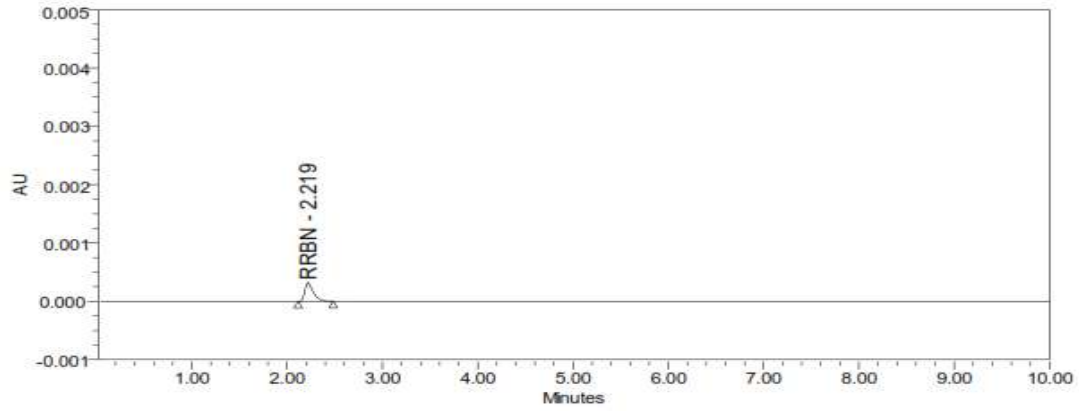

Fig. 4: Chromatogram for Limit of Detection

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank : 64 µV

Signal Obtained from LOD solution : 191 µV

S/N = 191/64 = 2.98

Acceptance Criteria:

S/N Ratio value shall be 3 for LOD solution.

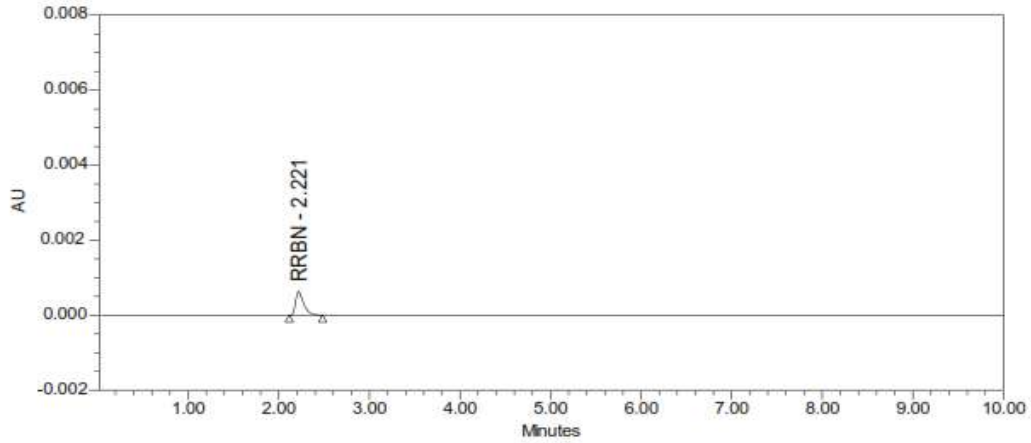

Fig. 5: Chromatogram for Limit of Quantification

Calculation of S/N Ratio

Average Baseline Noise obtained from Blank : 64 µV

Signal Obtained from LOQ solution : 638 µV

S/N = 638/64 = 9.97

Acceptance Criteria

S/N Ratio value shall be 10 for LOQ solution.

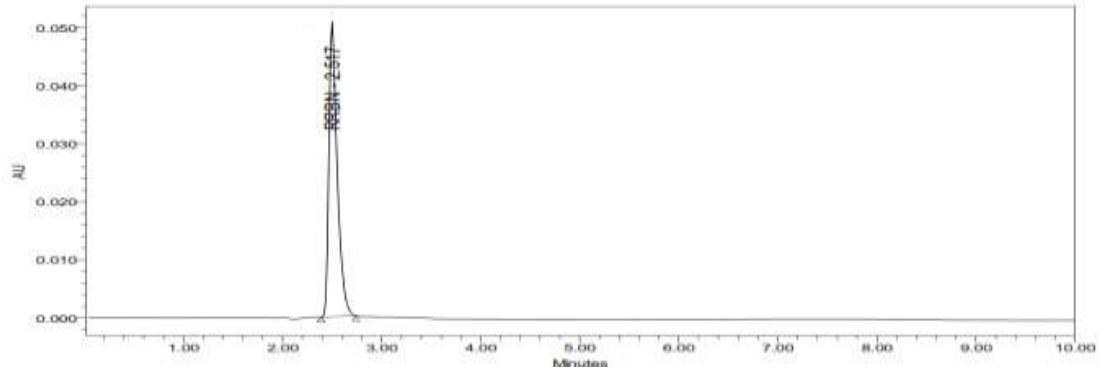

Fig. 6(a): Chromatogram for Robustness (less flow)

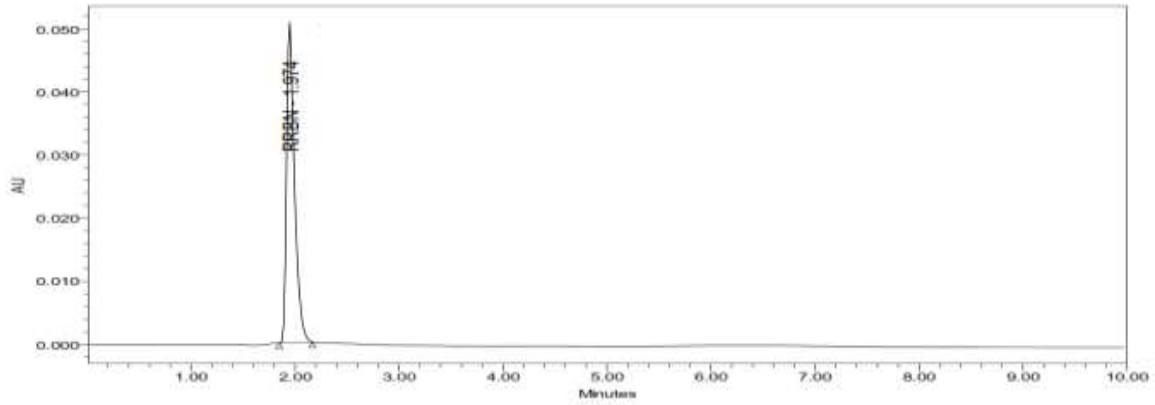

Fig. 6(b): Chromatogram for Robustness (more flow)

Table 4: System suitability results for Rivaroxaban

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	3639.37	1.55
2	1	3248.37	1.53
3	1.1	3386.38	1.54

***** Results for actual flow (1ml/min) have been considered from Assay standard.

B. The Organic composition in the Mobile phase was varied from 54% to 66%

Standard solution 75ppm of Rivaroxaban was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

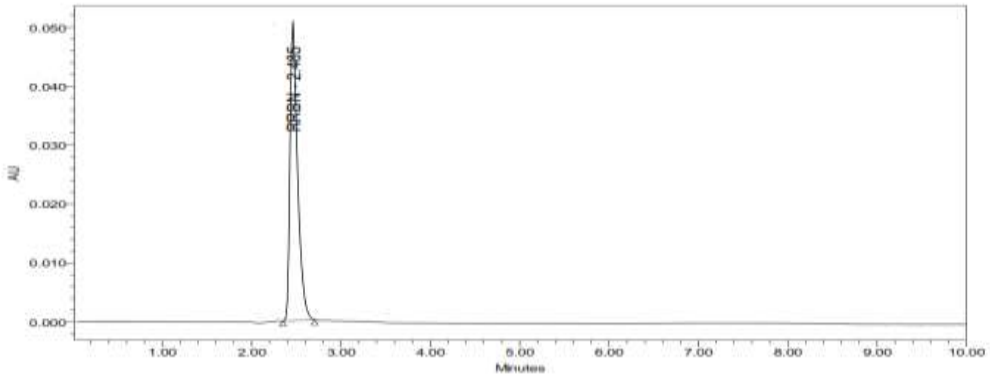

Fig. 7(a): Less Organic Composition

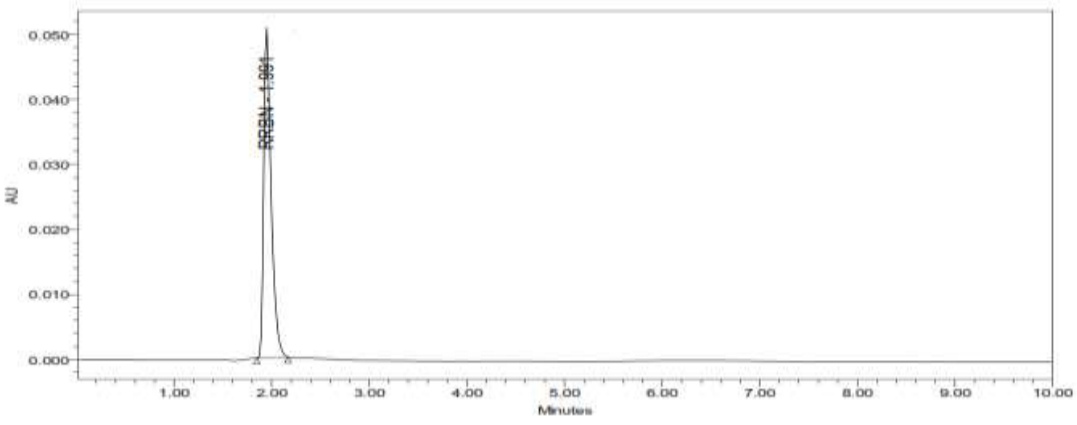

Fig. 7(b): More Organic Composition

Table 5: System suitability results for Rivaroxaban

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3674.67	1.55
2	*Actual	3248.37	1.53
3	10% more	3465.33	1.53

***** Results for actual Mobile phase composition (40:60) Buffer (ph-3): Acetonitrile has been considered from Accuracy stand

Table 6: Degradation results for Rivaroxaban

Sample Name	Rivaroxaban				
	Area	% Degraded	Purity Angle	Purity Threshold	Peak purity
Standard	346387				
Acid	316528	8.62	0.7539	1.250	Passes
Base	338212	2.36	0.208	1.252	Passes
Peroxide	324461	6.33	0.123	0.262	Passes
Thermal	340602	1.67	0.180	0.255	Passes
Photo	334402	3.46	0.168	0.253	Passes

Degradation Studies

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Rivaroxaban using the proposed method.

CONCLUSION

From the above experimental results it was concluded that, this newly developed method for the estimation of Rivaroxaban was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories.

REFERENCES

1. 1. S Roehrig; A Straub; J Pohlmann; T Lampe; J Pernerstorfer; KH Schlemmer; P Reinemer; E Perzborn. J MedChem, 2005, 48(1), 5900-5908.
2. V Shiva Shankar; M Gandhimathi; TK Ravi. IJPAR, 2015, 4(4), 406-410.
3. M Arun; Prajapathi Harsha; A Patel. Int J Pharm, 2015, 5(2), 610-613.
4. PSP Rao; VK Cholleti; VR Reddy. Int J Res Pharm Sci, 2015, 5(2), 17-24.
5. Hetal Jebaliya; Batuk Dabhi; Madhavi Patel; Yashwantsinh Jadeja; Anamik Shah. J Chemical Pharmaceutic Res, 2015, 7(10), 65-74.
6. Manjunatha Devagondanahalli Hadagali. Int J Adv in Pharmac Ana, 2015, 5(3), 65-68.
7. N Rajan; K Aner Basha. J phar res, 2014, 8(11), 1719-1725.
8. Burla sunitha venkata seshamamba; Peruri veera venkata Satyanarayana;ChandrabalaSekaran. Chem Sci Trans, 2014, 3(4),1546-1554.
9. Mustafa Çelebier; Tuba Reçber; Engin Koçak; Sacide Altınöz. Braz J Pharm Sci, 2013, 49(2).
10. Pinaz A Kasad. Int J PharmTech Res, 2013, 5(3), 1254-1263.
11. IB Lories; AA Mostafa; Girges. J Chromatogr Sep Tech, 2013, 4(8).
12. PVV Satyanarayana; Alavala Siva Madhavi. IJSID, 2012, 2(1), 226-231.
13. K Chandra sekhar;. Research Desk, 2012, 1(1), 24-33
14. ICH Guideline: Validation of Analytical Procedures, Text and Methodology, Federal registration publications, EU, 2005, 2-17.