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Analytical method of development and validation of rivaroxaban in bulk drug and pharmaceutical drugs from RP-HPLC

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Abstract

I have done research and achieved the method of development and validation of Rivaroxaban in bulk drug and pharmaceutical dosage form by RP-HPLC method. The analysis of drug carried out an agilent technique gradient system with auto injector, UV(DAD) and Gradient detector. Equipped with reverse phase (agilent) C18 column (4.6mm * 100mm; 2.5 um), 20 ul injection loop and UVD30D absorbance detector and running chemstration 10.1 software. Methanol and 0.1% formic acid (65:35).

Keywords: Validation; RP-HPLC method; UV(DAD); Gradient detector

1. Introduction

Rivaroxaban is a direct factor Xa inhibitor, an anticoagulant medication used to prevent and treat blood clots. It works by inhibiting the factor Xa, a key enzyme in blood clotting cascade, thereby reducing the risk of stroke and other complications associated with abnormal blood clotting. Rivaroxaban is orally active oxazolidine derivative. Its oral bioavailability is greater than 80% . Rivaroxaban is anticoagulant medication used to treat and reduce risk of blood clots.

1.1. Drug Profile

Chemical name is (S)-5-chloro-N-{[2-oxo-3-[4-(3-oxomorpholin-4-yl) phenyl]oxazolidin-5-yl]methyl} thiophene-2-carboxamide. It is anticoagulant medication having molecular formula C19H18ClN3O5S.

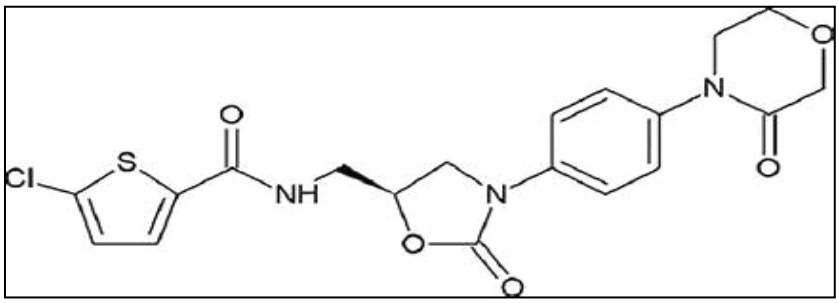

Figure 1 Structure of Rivaroxaban

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2. Material and methods

2.1. Chemical and Reagent

Methanol, 0.1% Formic acid, 0.15 OPA, Water

2.2. Instruments

The analysis of the drug was carried out on Agilent Tech. Gradient System With Auto injector, UV(DAD)(G13148,S.NO.DE71365875) and Gradient Detector, Quaternary Gradient pump((G130A)S.NO.D DE9180834),Equipped with Reverse Phase (Agilent) C18 column (4.6mm x 100mm; 2.5µm),a 20µl injection loop and UV730D Absorbance detector and running chemstration 10.1 software, UV spectroscopy, Ph meter, Balance, Sonicator.

2.3. Stock preparations

Stock I : Standard Sample Preparation:- Accurately weight and transfer 10 mg Rivaroxaban working standard into 10 ml volumetric flask as about diluents Methanol completely and make volume up to the mark with the same solvent to get 1000µg/ml standard (stock solution) and 15 min sonicate to dissolve it and the resulting stock solution 0.1ml was transferred to 10 ml volumetric flask and the volume was made up to the mark with mobile phase Methanol: (0.1 % formic Acid)Water, prepared in (6.0 ml MEOH: 4.0 ml (0.1 %Formic acid)WATER v/v)solvent.

Stock II : Tab solution Preparation:- Take 56.2 mgs in 10 ml Methanol i.e.= 1000 µgm/ml.

2.4. Linearity

Linearity of an analytical method is its ability to elicit test results that are directly or by a well defined mathematical transformation, proportional to the concentration of analyst in samples within a given range.

2.5. Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often the expressed as percent recovery by the assay of known added amounts of analyst.

The accuracy of an analytical method is determined by applying the method to analyzed samples, to which known amounts of analyst have been added. The accuracy is calculated from the test results as the percentage of analyst recovered by the assay.

2.6. Precision

Precision of an analytical method is the degree of agreement among Individual test results when the procedure is applied repeatedly to multiple Samplings of a homogenous sample. Precision of an analytical method is usually expressed as standard deviation or relative standard deviation. Also, the results obtained were subjected to one way ANOVA and within-day mean square and between-day mean square was determined and compared using F-test.

2.7. Repeatability

Precision of the system was determined with the sample. Two replicates of sample solution containing 50µg/ml of rivaroxaban was injected and peak areas were measured and %RSD was calculated it was repeated for two times result.

2.8. Robustness

The mobile phase composition was changed in (±1 ml/ min-1) proportion and the flow rate of Methanol in the mobile phase composition (±1 ml/ min-1) and the change in detection wavelength (±1 ml/ min-1) and the effect of the results were examined it was performed using 30µg/ml solution of Rivaroxaban in triplicate.

3. Results and discussion

Studies on the chromatographic behavior of Rivaroxaban:

3.1. Chromatogram of Trial 1

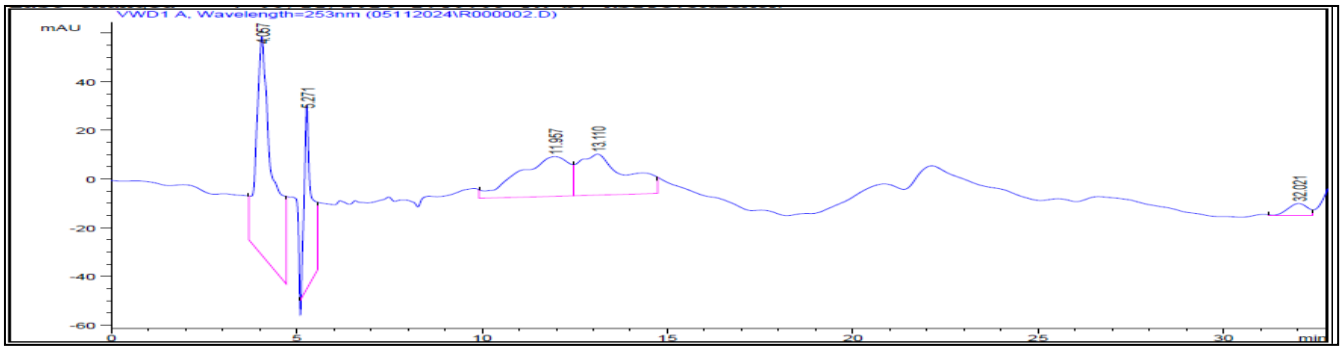

Figure 2 Chromatogram of Trial 1

Table 1 Result for Chromatogram of Trial 1

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	4.057	2761.7031	7902	0.48	0.0000
2	5.271	1065.8592	2614	0.48	2.45
3	11.957	1566.5213	280	2.72	4.08

3.2. Chromatogram of Trial 2

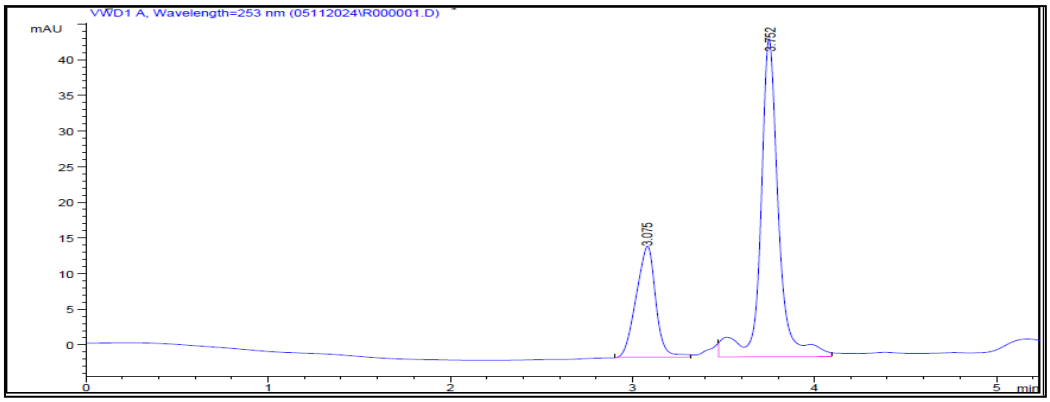

Figure 3 Chromatogram of Trial 2

3.3. Linearity

Linearity of an analytical method is its ability to elicit test results that are directly or by a well defined mathematical transformation, proportional to the concentration of analyst in samples within a given range, the Result are shown in; (Table No 2).

Table 2 Table for linearity

Sr No.	Concentration (µg/ml)
	Rivaroxaban
1	10
2	20
3	30
4	40
5	50

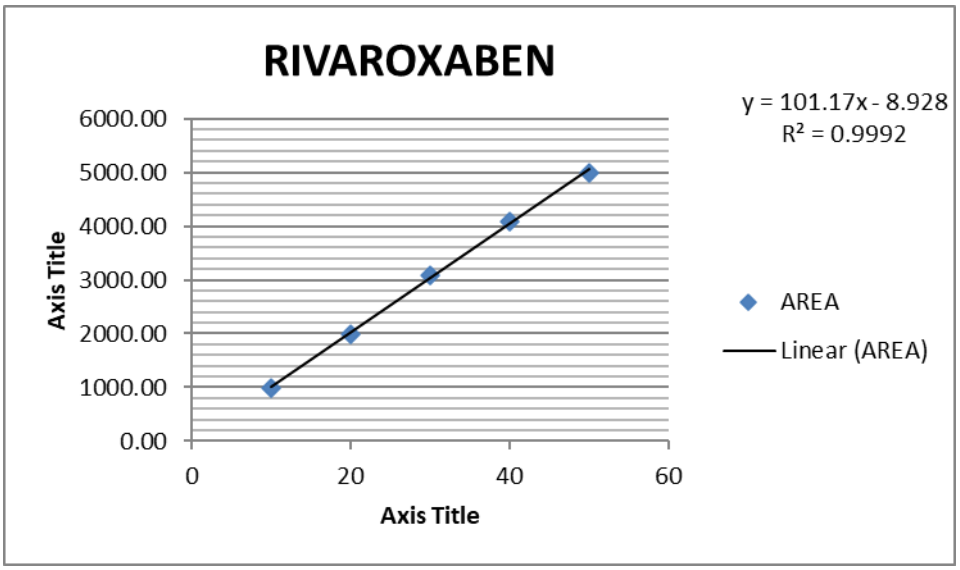

Figure 4 Chromatogram Of Rivaroxaban

3.4. Accuracy (recovery)

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often the expressed as percent recovery by the assay of known added amounts of analyte. The accuracy of an analytical method is determined by applying the method to analyzed samples, to which known amounts of analyte have been added. The accuracy is calculated from the test results as the percentage of analyte recovered by the assay, The Result are shown in; (Table 3). The accuracy of an analytical method is determined by applying the method to analyzed samples, to which known amounts of analyte have been added. The accuracy is calculated from the test results as the percentage of analyte recovered by the assay, The Result are shown in; (Table No: 3)

Table 3 Result of Recovery data for Rivaroxaban

Drug	Sr No.	Level (%)	Amt. taken (µg/ml	Amt. Added (µg/ml	Area.Mean* ± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean *± S.D.
RVK	1	80%	10	8	17.9± 0.027	7.95± 0.027	99.36±0.34
	2	100%	10	10	20.1±10.18	10.18±0.098	101.82± 0.98
	3	120%	10	12	22.1±0.009	12.19±0.009	101.61 ±0.07

3.5. Precision

Precision of an analytical method is the degree of agreement among Individual test results when the procedure is applied repeatedly to multiple Samplings of a homogenous sample. Precision of an analytical method is usually expressed as standard deviation or relative standard deviation. Also, the results obtained were subjected to one way ANOVA and within-day mean square and between-day mean square was determined and compared using F-test.

Table 4 Result of Intraday and Inter day Precision for Rivaroxaban

Concⁿ (µg/ml)	**Intraday Precision**			**Interday Precision**		
	Mean± SD	%Amt Found	%RSD	Mean± SD	%Amt Found	%RSD
10	1028.49±0.10	102.61	0.48	1030.5±3.01	101.05	0.29
20	2050.48±2.15	101.85	0.46	2054.19±9.01	101.15	9.01
30	3015.65±1.74	99.72	0.21	3016.83±1.98	99.17	0.07

3.6. Repeatability

Precision of the system was determined with the sample. Two replicates of sample solution containing 20 µg/ml of Rivaroxaban were injected and peak areas were measured and %RSD was calculated it was repeated for two times.

3.7. Detection Limit

Based on the S.D. of the response and the slope of calibration curve, the detection limit (DL) was calculated as,

$$DL = \frac{3.3\sigma}{S}$$

Where,
σ = the S.D. of the y-intercepts of regression lines.
S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve and S.D. was used should be calculated from the y-intercepts of regression line in calibration curve.

3.8. Quantitation Limit

Based on the S.D. of the response and the slope of calibration curve, the quantitation limit (QL) was calculated as,

$$QL = \frac{10\sigma}{S}$$

Where,
σ = the S.D. of the y-intercepts of regression lines.
S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve and S.D. was used should be calculated from the y-intercepts of regression line in calibration curve.

3.9. Robustness

The Robustness of a method is its ability to remain unaffected by small deliberate changes in parameters. To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate, wavelength on retention time and tailing factor of drug peak was studied.

The mobile phase composition was changed in(±1 ml/min-1) proportion and the flow rate was varied by of optimized chromatographic condition. The results of robustness studies are shown in Robustness parameters were also found satisfactory; hence the analytical method would be concluded.

Table 5 Result of Robustness Study of Rivaroxaban

Parameters	Conc.(µg/ml)	Amount of detected(mean ±SD)	%RSD
Mob-phase composition 64+36ml) Methanol + 0.1% (Buffer)water	30	2925.3±14.27	0.49
Mob-phase composition (66ml+34ml) Methanol + 0.1%(Buffer)water	30	2928.40±6.85	0.23
Wavelength change252nm	30	2866.4±27.56	0.96
Wavelength Change 254nm	30	1224.41±4.24	0.35
Flow rate change(0.9ml)	30	3097.1±2.12	0.07
Flow rate change(1.1 ml)	30	2953.20±7.02	0.24

Wavelength Change 252 nm

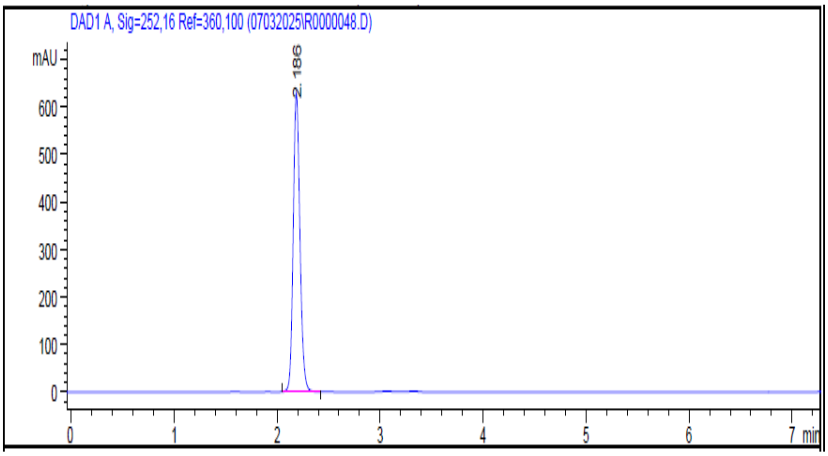

Figure 5 Chromatogram of wavelength change 252 nm

Table 6 Result for Chromatogram of wavelength change 252 nm

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	2.186	2846.9432	5789	0.83	0.0000

3.10. System suitability parameters :(Repeatability)

To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of Rivaroxaban system suitability parameters were studied.

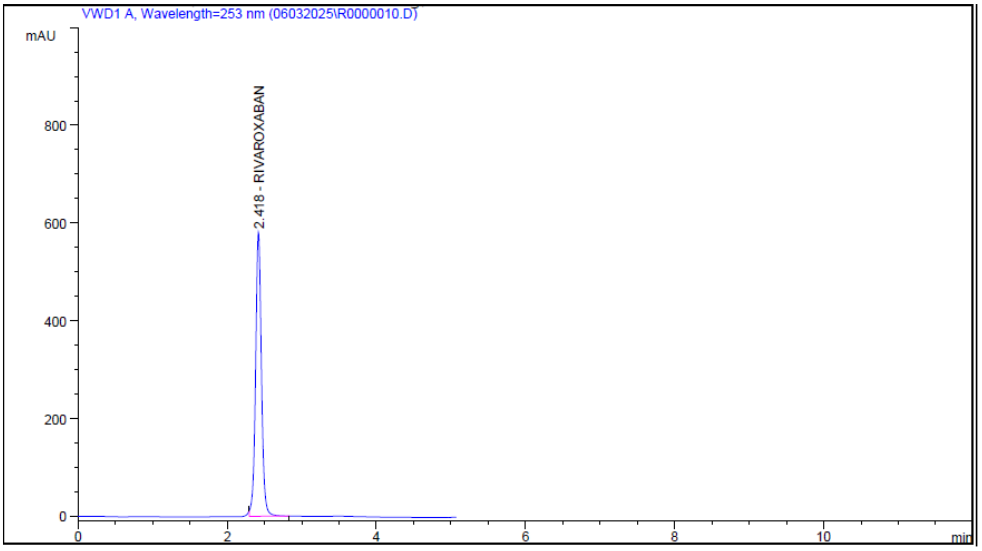

Figure 6 Chromatogram of System suitability No- 1 hr

Table 7 Result for Chromatogram of System suitability No- 1hr

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	2.418	3012.5266	5051	0.90	0.0000

3.11. Analysis of marketed formulation

To determine the content of Rivaroxaban in marketed Tablets (20 Tablets were weighed, and average weight was calculated. Tablets were triturated and powder equivalent to 56.2 mg of Rivaroxaban was weighed. The drug was extracted from the Tablet powder with 10 mL Methanol. To ensure complete extraction it was sonicated for 15 min. 0.2 mL of supernatant was then diluted up to 10 mL with mobile phase. The resulting solution was injected in HPLC and drug peak area was noted. (Fig .5, 6)

Regression equation was generated using peak areas of standard solutions. Using the regression equation and peak area of the sample the amount of Rivaroxaban in the sample was calculated. The amount of Rivaroxaban per Tablet was obtained from the regression equation of the calibration curve as described in analysis of Tablet formulation (Table No:8)

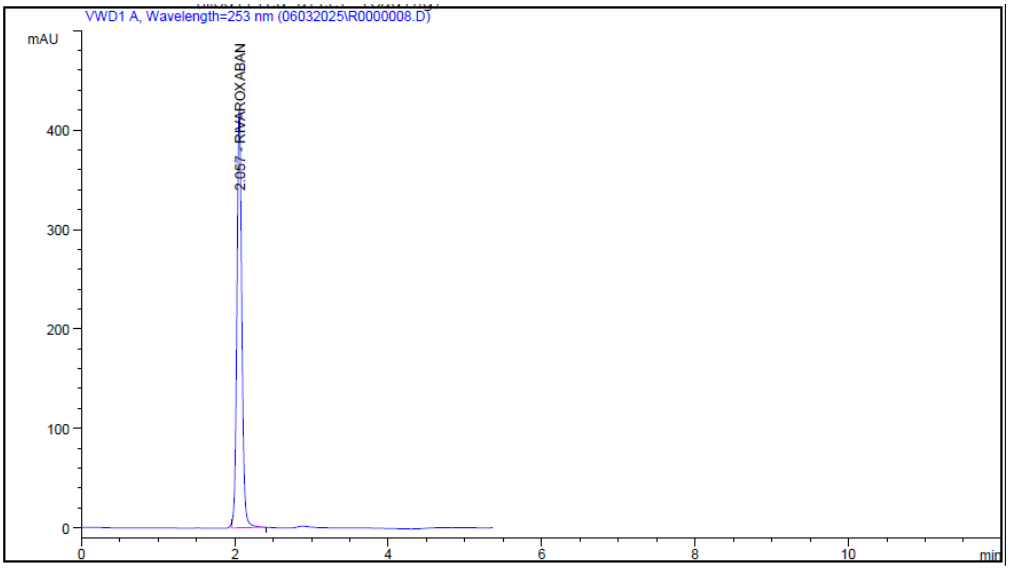

Figure 7 Chromatogram for Marketed Formulation

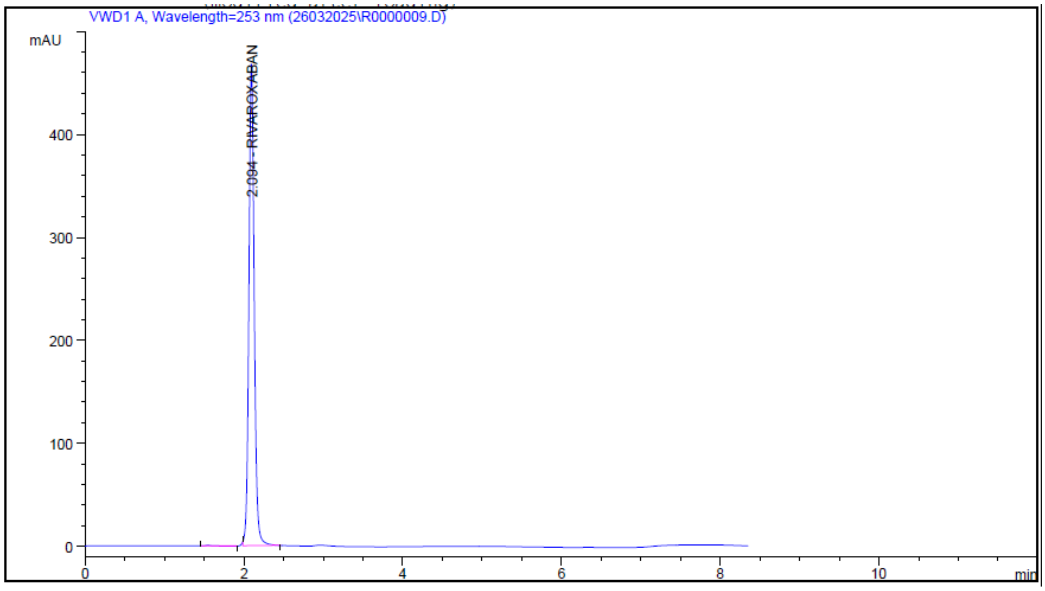

Figure 8 Chromatogram for Marketed Formulation

Table 8 Analysis of marketed formulation

Sr.no	Amount present in mg	Area(I)	Amount found in mg	% Label claim
	RVK	RVK	RVK	RVK
1	20	1998.366	19.67792	98.39
2	20	2000.113	19.6952	98.48
Mean	-	1999.24	19.69	98.43
SD	-	1.236	0.012	0.061
%RsD	-	0.062	0.062	0.062

4. Conclusion

In this research a simple HPLC method was developed for determination of Rivaroxaban in bulk drug and fixed Pharmaceutical formulation. The approach offers several advantages, including its simplicity, preciseness, ease of use and shorter run times which is particularly time saving when dealing with large number of samples. The validation tests demonstrated a method with a wide linear range, acceptable precision and accuracy and reliable sensitivity. The developed method allows for easy, selective, sensitive and specific analysis of Rivaroxaban, making it suitable for routine use in pharmaceutical quality control.

Compliance with ethical standards

Disclosure of conflict of interest

Author declares no conflict of interest in constructing this manuscript

References

[1] Jeffery G.H, Bassett J, Mendham J, And Denney R C., Vogel's Textbook Of Quantitative Chemical Analysis, 5th Ed., Addison Wesley Longman Inc, Singapore, 2001, 4-5.

[2] Erwing G.W., Instrumental Methods Of Chemical Analysis, 2nd Ed., Mcgraw Hill Publishing Company Inc, 1960, 1-3.

[3] Skoog D.A., Holler F.J. And Nieman T.A., Principles Of Instrumental Analysis, 5th Ed., Thomson Brooks/Cole Asia Pvt. Ltd, Singapore, 2004, 4-7.

[4] Lalitha N., Sanjay P.N., Vyshak M.G., Kadri U. And Trop. J. Pharma. Stability-Indicating Reverse Phase Hplc Method For The Determination Of Cefazolin Res, 9(1), 2010, 45-50.

[5] Willard R.H., Merritt L.L., Dean L.A., And Settle F.A., Instrumental Methods Of Analysis, 7,C.B.S. Publishers, New Delhi, 1986, 177-178.

[6] Skoog D.A., Holler F.J. And Nieman T.A., In Principles of Instrumental Analysis, 5, Thomson Brooks/Cole, 1998, 329-335.

[7] Brown R.P., Reversed-Phase High Performance Liquid Chromatography, Theory, Practice And Biomedical Applications, 1982, 10-20.

[8] Settle F., In; Handbook Of Instrumental Techniques For Analytical Chemistry, Pearson Education Pte. Ltd., Delhi, 2004, 154-156.

[9] Kasture A.V., Mahadik K.R. And More H.N., Instrumental Methods 14(2), Niraliprakashan, Pune, 2006,44-55.

[10] Sethi, P.D., In Hplc 'High Performance Liquid Chromatography', Quantitative Analysis Of Pharmaceutical Formulations, 1, Cbs Publishers And Distributors, New Delhi, 2001, 3-72, 116-120.

[11] Beckett A.H. And Stenlake J.B., In Practical Pharmaceutical Chemistry, 4(2),

[12] Cbs Publishers And Distributors, New Delhi, 2002, 275-278, 281-300.

[13] Christian G.D., In; Analytical Chemistry, 6. Jhon Wiley And Sons, 2004, 1-7.

[14] Connors, K.A., In; A Textbook Of Pharmaceutical Analysis, 3, Jhon Wiley And Sons, 1999, 196-198.

[15] Brown R.P., Reversed-Phase High Performance Liquid Chromatography, Theory, Practice And Biomedical Applications, 1982, 10-20.