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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF CEFTOLOZANE AND TAZOBACTAM BY RP-HPLC METHOD IN PURE AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of Ceftolozane and Tazobactum in Pharmaceutical dosage form. Chromatogram was run through Std ODS 250mm x 4.6 mm, 5m. Mobile phase containing Buffer (Ortho Phosphoric Acid), Acetonitrile in the ratio of (60:40) was pumped through column at a flow rate of 1ml/min. Temperature was maintained at 30°C. Optimized wavelength for Ceftolozane and Tazobactum was 210nm. Retention times of Ceftolozane and Tazobactum were found to be 2.335min and 3.850min. % RSD of the Ceftolozane and Tazobactum were and found to be 0.9 and 0.5 respectively. % Recover was Obtained as 99.30% and 99.67% for Ceftolozane and Tazobactum respectively. LOD, LOQ values obtained from regression equations of Ceftolozane and Tazobactum were 0.02, 0.03 and 0.07, 0.08, respectively. Regression equation of Ceftolozane is y =16395.x + 11260 and Tazobactum is y = 18140x + 5689. Regression co-efficient was 0.999. The method developed was simple and economical that can be adopted in regular Quality control test in Industries.

KEYWORDS

Ceftolozane, Tazobactam, Ortho phosphoric acid and ACN.

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INTRODUCTION[1]

Russian botanist Tswett introduced chromatography as a separation technique. He explained in detail the separation of pigments, the coluored substances by filtration through column, followed by developments with pure solvents. Chromatography is method of separating mixtures and identifying their components i.e. it is a separation method that exploits the differences in partitioning behaviour of analyte between a mobile phase and a stationary

phase to separate components in a mixture. Chromatography may be preparative or analytical.

High Pressure Liquid Chromatography (HPLC) is a separation technique that can be used for the analysis of organic molecules and ions. HPLC is based on different mechanisms like adsorption, ion exchange and partition or size exclusion, based on the type of stationary phase used. HPLC involves a solid stationary phase, normally packed inside a stainless-steel column, and a liquid mobile phase. Separation of the components based on the difference in the relative distribution ratios of the solutes between the two phases.

The present work is under taken with an aim to develop a simple, precise, accurate method for the simultaneous estimation of Ceftolozane and Tazobactam in Pure and Pharmaceutical dosage form by using RP-HPLC method[2].

The main objective of the present work is to develop a method and validate the work by a suitable high precision and accurate analytical method for the Simultaneous estimation of Ceftolozane and Tazobactam in Pure and Pharmaceutical dosage form by using RP-HPLC method.

MATERIAL AND INSTRUMENTS

All the materials and equipments used in this work are tabulated in Table No.1, 2, 3 and 4.

METHODOLOGY

EXPERIMENTAL WORK

[3,4]The objective of this experiment was to optimize the method for simultaneous estimation of Ceftolozane and Tazobactum from the literature survey made. So here the trials mentioned de scribe show the optimization was done.

Optimization of Chromatographic Conditions for the Estimation of Ceftolozane and Tazobactam

Selection of wavelengthbyuv-spectroscopy[5,6]

From the UV-visible spectrophotometric results, a detection wavelength of 210nm was selected. At this wavelength they showed maximum absorbance with good peak intensity, good peak shape and height was observed.

Selection of Chromatographic Method

Proper selection of method depends upon the nature of the sample (ionic/ionisable/neutral molecules), its molecular weight and solubility. The drug selected in the present study is polar in nature and hence Reverse phase method may be used. The RP-HPLC selected for the initial separation Because of its simplicity and suitability

METHOD DEVELOPMENT

Preliminary studies[7,8,9]

As a starting point for method developments following preliminary studies are performed for the sample.

Preparation of solutions

Preparation Buffer: (0.1%OPA)

1ml of Ortho phosphoric acid solution in a 1000ml Volumetric flask, add about 100ml of milli-Q water and final volume was made up to 1000 ml with milli-Q water.

Mobile phase

Buffer and Acetonitrile taken in the ratio 60:40

Diluent

Water and Acetonitrile

Standard Preparation: (100µ g/ml Ceftolozane& 50µ g/ml Tazobactam)

Accurately Weighed and transferred 25mg and 12.5mg of Ceftolozane and Tazobactam working Standards into a 25ml clean dry volumetric flask respectively, add 10ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solutions, 1ml was pipette out in to a 10ml volumetric flask and then make up to the final volume with diluent.

Sample Preparation

One vial powder was weighed and then the weight equivalent to 1.85gm of ceftolozane and Tazobactam was transferred into a 1000ml volumetric flask, 500ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipette out into a 10ml volumetric flask and made up to 10ml with diluents.

Method development Experimental Trials

Trial-1

Observation: Two peaks were observed. Ceftolozane was eluted at 2.529 and tazobactum eluted at 2.889. The peaks recorded were found to be not good and Resolution was less

Trial-2

Observation: Ceftolozane eluted at 2.325 and tazobactum eluted at 3.802. Ceftolozane peak record was founded to be not good and tailing was not passed. So further trails are carried out.

Trial-3

Observation: Ceftolozane eluted at 2.261 and tazobactum eluted at 3.150. Ceftolozane peak record was founded to be not good and tailing was not passed. So further trails are carried out.

Trial-4

Observation: Ceftolozane eluted at 2.546 and Tazobactum eluted at 5.051.Peaks shapes was good but resolution of chromatograms was not satisfactory. So further trails are carried out.

Trial-5

Observation: Ceftolozane eluted at 2.315 and Tazobactum at3.685.Peak shape was good.

Optimization of separation conditions

Effect of Ratio of mobile phase

The mobile phase of Acetonitrile, Ammonium Acetate and Ortho phosphoric acid, 40:60, 45:55, 50:50, 70:30 were tried and chromatograms were recorded at 210 nm with flow rate of1ml/min. At the ratio of 60:40 of Acetonitrile: Orthphopharic acid selected as the Ideal ratio for estimation of ceftolozane and Tazobactam.

Effect of flow rate

Keeping the mobile phase 60:40 of Orthophosphric acid: Acetonitrile and Chromatogram were recorded at flow rate of 1ml/min. At this flow rate, the peaks were with good resolution. So 1ml/min was kept constant for the analysis.

Optimized Chromatographic Conditions

Based on the above studies, the following Chromatographic conditions were finally optimized for the simultaneous estimation of Ceftolozane and Tazobactam.

METHOD VALIDATION

After satisfactory development of method, it was subjected to method validation as per ICH guidelines.

Method validation study includes various parameters like,

Linearity
Assay
Accuracy
Precision
Specificity/ Selectivity
Robustness
System Suitability
Degradation Studies
Limit of Detection
Limit of Quantification

RESULTS AND DISCUSSION

Method development Trails

Conclusion

Retention time of the peak is improved. Resolution of the peak is good. Peak intensity is good. Hence, this method is considered as the final optimized method in the development process.

Summary

From the reported literature, there were few methods established for the determination of Ceftolozane and Tazobactam in individual and in combination with other drug. It was concluded that there was no method reported for the simultaneous estimation of the Ceftolozane and Tazobactam, which promote to pursue the present work. The scope and objective of the present work is to develop and validate a new simple RP-HPLC method for simultaneous estimation of Ceftolozane and Tazobactam.

In simultaneous RP-HPLC method development, Waters HPLC with PDA detector and column used is ODS, 250×4.6 mm, 5m. Injection volume of 10µl is injected and eluted with the mobile phase selected after optimization. Acteonitrile and Buffer (Ortho phosphoric Acid) in the ratio of 40:60 was found to be ideal. The flow rate was found to be optimized at 1 ml/min. Detection was carried out at 210 nm. Quantitation was done by external standard method

with the above mentioned optimized chromatographic condition. This system produced symmetric peak shape, good resolution and reasonable retention times of Ceftolozane and Tazobactam were found to be 2.315 and 3.685 minutes respectively.

The Ceftolozane and Tazobactam showed linearity in the range of 25ppm to 150ppm and 12.5ppm to 75ppm. The slope, intercept and correlation coefficient(s) were found to be 16359, 11260 and 0.999 for Ceftolozane and 18140, 5684 and 0.999 respectively for Tazobactam which indicates excellent correlation between response factor Vs concentration of standard solutions.

Precision of the developed method was studied. The % RSD values for precision was found to be within the acceptable limit, which revealed that the developed method was precise. The developed method was found to be robust. The % RSD value for percentage recovery Ceftolozane and Tazobactum was found to be within the acceptance criteria.

The results indicate satisfactory and accurate method for simultaneous estimation of the Ceftolozane and Tazobactam.

Stability indicating property of analyte was performed by forced degradation studies. Ceftolozane and Tazobactam were subjected to various stress conditions like Acid, Alkaline, Peroxide, Thermal and Photolytic conditions. Result revealed no change in drug solution at Photolytic, Neutral and Thermal. So % Recovery is very close to 100% which indicates the drug stability. The analyte shows slightly degradation with Acid, Alkaline and Peroxide Stress Conditions.

There is no simultaneous estimation method that has been reported so far for Ceftolozane and Tazobactam formulation. Hence in the present investigation an attempt has been made to develop the study by RP-HPLC method.

Table No.1: Drug (Brand Used)

S.No	Name	Label Claim	Brand Used
1	Ceftolozane	1g (equivalent to1.47gofCeftolozanesulphate)	Zerbaxa (power for injection)
2	Tazobactam	0.5g (equivalent to 0.537gmoftazobactamsodium)	

Table No.2: Chemical Reagents

S.No	Name	Grade	Make
1	Acetonitrile	HPLC	Rankem
2	Water	HPLC	Rankem
3	Methanol	HPLC	Rankem
4	Orthphosphoric acid	HPLC	Rankem

Table No.3: Instruments Used

S.No	Name of Instruments	Make	Model
1	Analytical Balance	Sartorius	BSA2245-CW
2	Ultra Sonicator	Esquire Biotech Ltd	Fast cleaner
3	UV-Visible	Lab India	UV-3200

Table No.4: Equipment's used

S.No	Name	Make	Model
1	HPLC	Waters	Waters 2965 HPLC(Empower)
2	Detector	Detector(PAD)	2996

Table No.5: Solubility test Drug-A

S.No	Drug–A	Description	Solvent Used	Result
1	Ceftolozane	Weighaccurately1.0gmofthesampleinto asolubilitytesttubeand add30ml of following solvents. Place the lid and shake well, if necessary, sonicated for10minutes. Test for the solubility of the sample.	Water	Freely soluble
			Acetonitrile	Freely soluble
			Methanol	Sparingly soluble
			Ethanol	Very slightly soluble

Table No.6: Solubility test Drug–B

S.No	Drug-B	Description	Solvent Used	Result
1	Tazobactam	Weighaccurately1.0gmofthe sample into a solubility test tube and add30ml of following solvents. Place the lid and shake well, if necessary, sonicate for10minutes. Test for the solubility of the sample.	Water	Freely soluble
			Acetonitrile	Freely soluble
			Methanol	Sparingly soluble
			Acetone	Sparingly soluble

Table No.7: HPLC Parameters for Trials

S.No	Parameter	Trail 1	Trail 2	Trail 3	Trail 4
1	Stationary phase (column)	BDS (250×4.6mm, 5m)	BDS 250×4.6mm, 5m	ODS 250×4.6mm, 5m	ODS 250×4.6mm, 5m
2	Mobile Phase A	Buffer (Ammonium Acetate)	Buffer (OrthoPhosphric Acid)	Buffer (OrthoPhosphric Acid)	Buffer (OrthoPhosphric Acid)
3	Mobile Phase B	Acetonitrile	Acetonitrile	Acetonitrile	Acetonitrile
4	Elution Mode	Isocratic	Isocratic	Isocratic	Isocratic
5	Mobile Phase ratio	40:60	45:55	50:50	70:30
6	Flow rate	1ml/min	1ml/min	1ml/min	1ml/min
7	Detector wavelength	210nm	210nm	210nm	210nm
8	Detector used	PDA	PDA	PDA	PDA
9	Column temperature	$30^{O}C$	$30^{O}C$	$30^{O}C$	$30^{O}C$
10	Injection volume	10µl	10µl	10µl	10µl
11	Runtime	20min	10min	10min	10min

Table No.8: HPLC parameters for Optimized method

S.No	Parameters	Description
1	Stationary Phase (column)	ODS250×4.6mm,5m
2	Mobile Phase A	Buffer (OrthoPhosphric Acid)
3	Mobile Phase B	Acetonitrile
4	Elution Mode	Isocratic
5	Mobile Phase Ratio	60:40
6	Flow Rate	1ml/min
7	Detector Wavelength	210nm
8	Detector Used	PDA
9	Column Oven Temperature	30^{0}
10	Injection volume	10µl
11	Runtime	10min

Table No.9: Trial-1Valuesof Ceftolozane and Tazobactam

S.No	Name of the peak	Retention time (min)
1	Ceftolozane	2.528
2	Tazobactum	2.889

Table No.10: Trial-2: Values of Ceftolozane and Tazobactam

S.No	Name of the peak	Retention time (min)
1	Ceftolozane	2.325
2	Tazobactum	3.802

Table No.11: Trial-3: Values of Ceftolozane and Tazobactam

S.No	Name of the peak	Retention time (min)
1	Ceftolozane	2.261
2	Tazobactum	3.150

Table No.12: Trial-4: Values of Ceftolozane and Tazobactam

S.No	Name of the peak	Retention time(min)
1	Ceftolozane	2.546
2	Tazobactum	5.051

Table No.13: Optimized Parameters

S.No	Name	Retention time	Peak area	Resolution	Tailing factor
1	Ceftolozane	2.315	1597142	8.7	1.6
2	Tazobactum	3.685	983669		1.5

Table No.14: Complete data of results obtained and Acceptance

S.No	Parameters	Obtained Results		Accepted Limits
		Ceftolozane	Tazobactam	
1	Retention Time	2.315min	3.685min	
2	Efficiency	2918	5240	NLT3000
3	%RSD	0.35	0.37	NMT2.0
4	Accuracy	100.2	100.04	98%-102%
5	Linearity(r^2)	0.999	0.999	0.999-1
6	LOD	0.02	0.03	
7	LOQ	0.07	0.08	
8	Assay	98.5%	99.57%	98%-102%

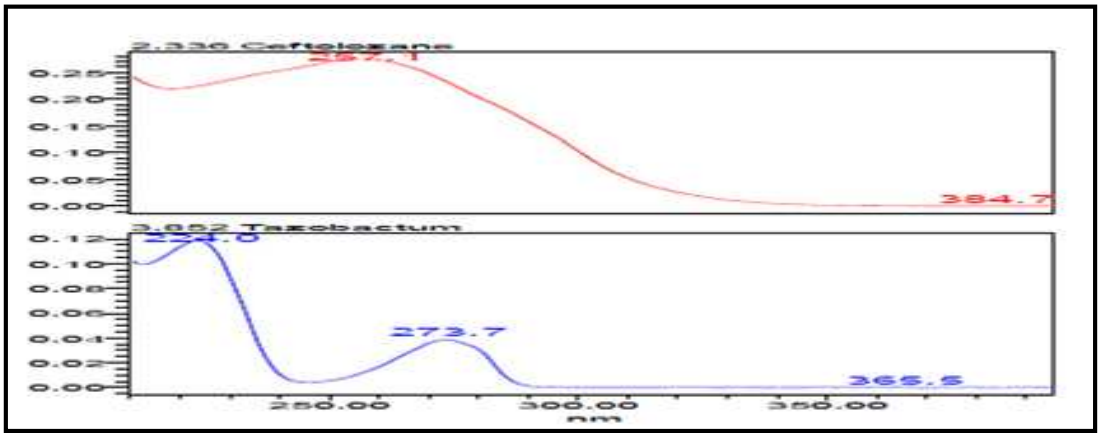

Figure No.1: UV Graph

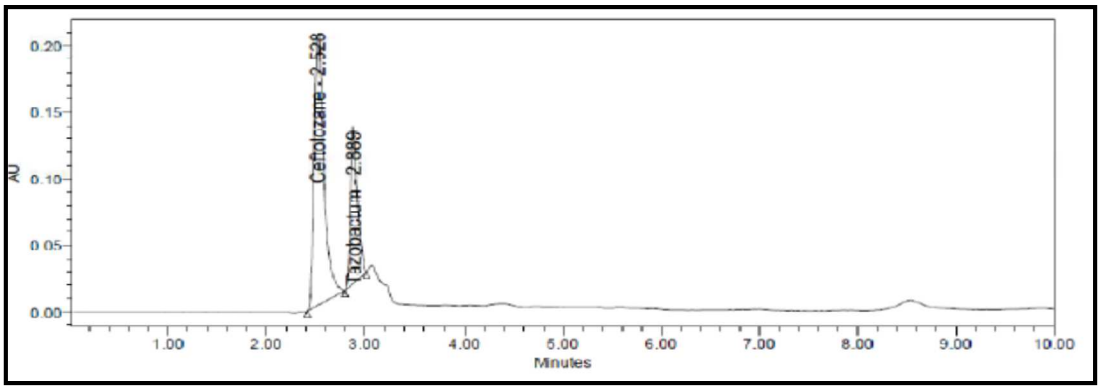

Figure No.2: Trial Chromatogram-1

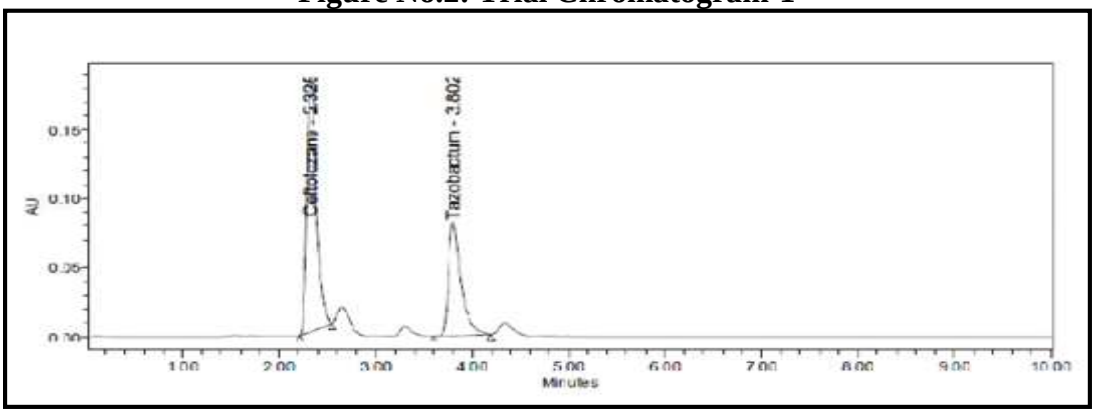

Figure No.2: Trial Chromatogram-2

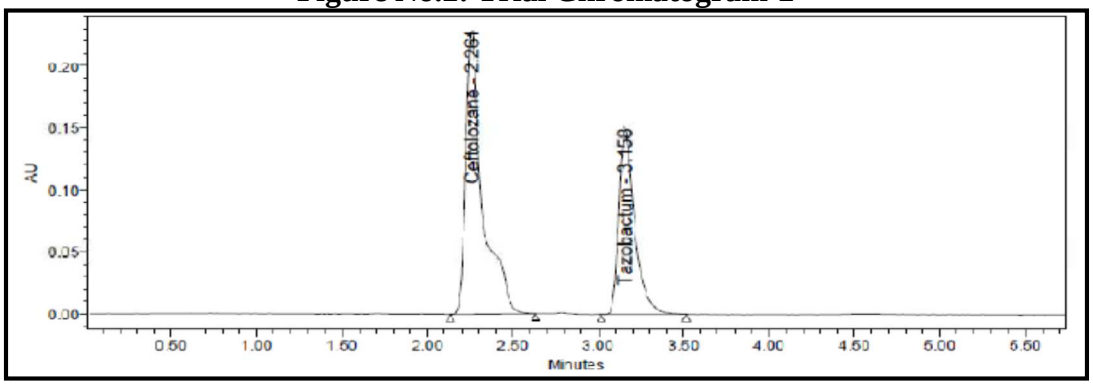

Figure No.3: Trial Chromatogram-3

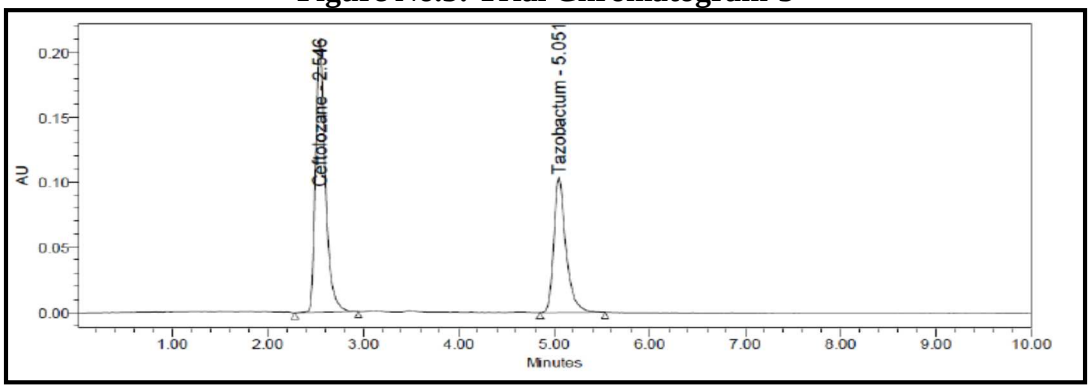

Figure No.4: Trial Chromatogram-4

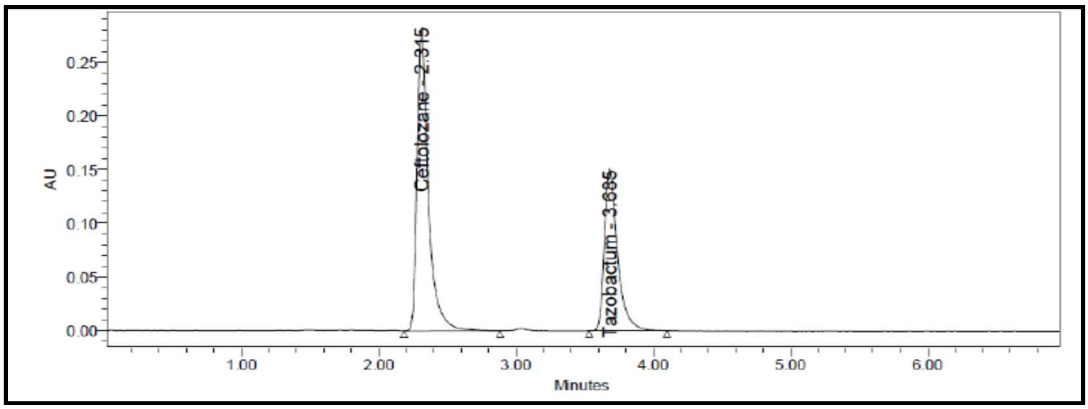

Figure No.5: Trial Chromatogram-5

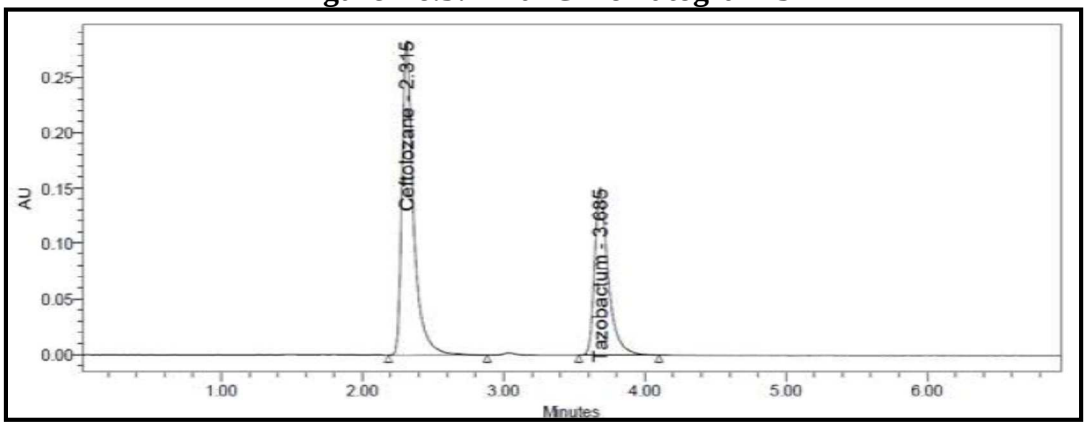

Figure No.6: Optimized Chromatogram

CONCLUSION

The objective of present work is to develop new validated RP-HPLC method for selected Pharmaceutical Dosage form and apply to stability indicating Assay. In the present work the RP-HPLC method development was done for Ceftolozane and Tazobactam using Mobile phase A and B containing Acetonitrile and Buffer (Orthophosphoric Acid) in the ratio of 40:60 and detection was performed at 210nm with are tention time of 2.135 min and 3.685min.

The method was validated for all validation parameters as per ICH guidelines. The linearity values of given method with respect to r2 value is 0.999, found was within acceptable limits. The % RSD for Intra and Inter day precision was < 2%. The accuracy of method was validated by recovery studies and was found to be significant and under specification limits, within acceptable range 98-102%. The method also passes the specifications for robustness parameters. A stability study on ceftolozane and Tazobactam were carried out and an efficient HPLC method for quantification and identification of its degradation products in Pharmaceutical dosage form was developed and validated. The results of stress testing of Pharmaceutical, undertaken according to ICH guidelines, revealed that degradation products were formed under acidic, alkaline, oxidation conditions. The method developed was economic as compared to previous methods and stability indicating. Hence it can be used for the routine analysis of Ceftolozane and Tazobactam in their combine dosage form in quality control laboratory and stability studies.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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