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Analytical Method Development and Validation of Ceftazidime Pentahydrate and Tazobactam Sodium by RP-HPLC Method in Bulk and Dosage forms.

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ABSTRACT

To develop a simple, accurate, precise and economical RP-HPLC method asper ICH guidelines for the simultaneous estimation of Ceftazidime pentahydrate and Tazobactam sodium in bulk and dosage forms. The chromatographic separation was performed on Octa Decyl Silane 250mm x 4.6 mm, 5µ using Ortho phosphoric acid: Acetonitrile (53:47(v/v)) as a mobile phase at a flow rate of 1.0 ml/min and detection of both the eluents was carried out by Photo Diode Array Detector. The Retention time of Ceftazidime pentahydrate and Tazobactam sodium were found to be 2.2 min. and 3.5 min respectively. Method was found to be linear over the range of 25-150µg/ml for Ceftazidime pentahydrate and 3.125-18.75 µg/ml for Tazobactam sodium. Percentage recovery of Ceftazidime pentahydrate and Tazobactam sodium was found to be 99.6% and 99.10% respectively. The percentage purity is 99.65% and 98.75% for Ceftazidime pentahydrate and Tazobactam sodium. The Limit of Detection of Ceftazidime pentahydrate and Tazobactam sodium is 0.34 µg/ml and 0.67 µg/ml and Limit of Quantification of Ceftazidime pentahydrate and Tazobactam sodium is 1.04 µg/ml and 2.0 µg/ml respectively. In the stress degradation studies, that Ceftazidime pentahydrate and Tazobactam sodium showed no degradation in UV and Water and degradation was found in acid, base, peroxide and thermal conditions. A stability-indicating HPLC method was developed for the simultaneous determination of Ceftazidime pentahydrate and Tazobactam sodium and validated as per ICH guidelines. The proposed method can be applied for routine quality control analysis of Ceftazidime pentahydrate and Tazobactam sodium in bulk and in Pharmaceutical dosage form.

Keywords: Ceftazidime pentahydrate, Tazobactam sodium, RP-HPLC, Photo Diode Array Detector.

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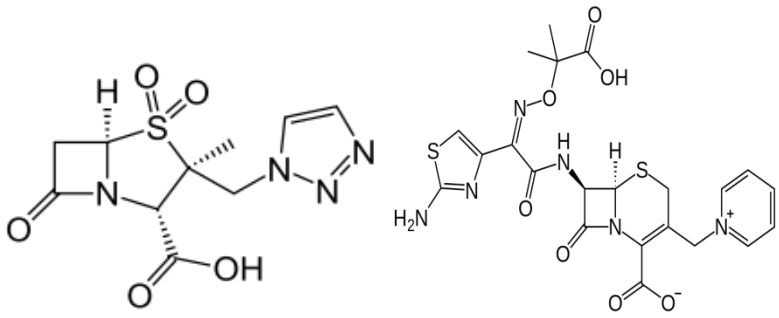
INTRODUCTION

Ceftazidime pentahydrate chemically is (6*R*,7*R*,*Z*)-7-(2-(2-aminothiazol-4-yl)-2-(2-carboxypropan-2-yl-oxyimino)acetamido)-8-oxo-3-(pyridinium-1-ylmethyl)-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylate pentahydrate.

Ceftazidime pentahydrate belongs to the cephalosporin class of antibacterial drugs. It is a broad-spectrum antibacterial derived from Cefaloridine and used especially for Pseudomonas and other gram-negative infections in debilitated patients.

The bactericidal action of Ceftazidime results from inhibition of cell wall biosynthesis and is mediated through binding to penicillin-binding proteins (PBPs). Ceftazidime is an inhibitor of PBPs of Peudomona aeruginosa (e.g., PBP1b, PBP1c, and PBP3) and E. coli (e.g., PBP3) [1,2].



Chemical structure of Tazobactam sodium and Ceftazidime pentahydrate

Tazobactam chemically is [2S-(2a,3b,5a)-3-methyl-7-oxo-3-(1H-1,2,3-triazol-1-ylmethyl)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide sodium salt.

Tazobactam is a antibacterial penicillin derivative which inhibits the action of bacterial beta-lactamases.Tazobactam broadens the spectrum of piperacillin by making it effective against organisms that express beta-lactamase and would normally degrade piperacillin [3]

The literature survey revealed that a number of methods being reported for the estimation of Ceftazidime pentahydrate [4] and Tazobactam sodium [5-11] individually but only one method has been reported on the combination by RP-HPLC with mobile phase contains phosphate buffer, acetonitrile and tetrahydrofuran in the ratio of 50:30:10 but tetrahydrofuran is more expensive.In our present work we have developed method with mobile phase having two solvents which are economical, retention time was also reduced than previous method andsimple and reliable RP-HPLC method for the estimation of Ceftazidime pentahydrate and Tazobactam sodium in bulk and pharmaceutical dosage forms.

MATERIALS AND METHODS

Instrumentation

HPLC instrument used was WATERS HPLC 2965 SYSTEM with Auto Injector PDA Detector. Software used is Empower, Sonicator (Ultrasonic sonicator),pH meter (Thermo scientific), Micro balance (Sartorius).

Chemicals and reagents

Ceftazidime pentahydrate and Tazobactam sodium were obtained as a gifted samples from Spectrum Labs, Hyderabad, India.Combined formulation, Combitaz (Lupin Pharmaceuticals Limited) containing Ceftazidime pentahydrate (1g) and Tazobactam sodium (125mg)obtained from pune, India.Milli-Q water, Ortho Phosphoric acid, Acetonitrile, were purchased from Rankem , Hyderabad, India and all the reagents are of HPLC grade.

Methodology [12]

Solubility:

According to literature, Ceftazidime pentahydrate is freely soluble inmethanol, Acetonitrile, waterand Tazobactam sodium is soluble in water. Both Ceftazidime pentahydrate and Tazobactam sodium are soluble in Acetonitrile and Water. Several dilutions of Acetonitrile were made for solubility of Ceftazidime pentahydrate and Tazobactam sodium. Finally Acetonitrile was chosen as solvent for present work.

Preparation of mobile phase Buffer

1 ml of Ortho phosphoric acid was taken in a 1000ml of volumetric flask adds about 900ml of milli-Q water added and degasses to sonicate and finally make up the volume with water, finally the pH was adjusted to2.8.

Standard Preparation:

Accurately Weighed and transferred 50mg and 25mg of Ceftazidime and Tazobactum working Standards into a 50ml and 100ml clean dry volumetric flask respectively, add 30ml and 20ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solutions, 1ml and 0.5ml was pipette out in to a 10ml volumetric flask and then make up to the final volumewith diluent. So that the concentration of Ceftazidime pentahydrate is 100µg/ml and Tazobactam sodium is 12.5µg/ml.

Sample Preparation:

One vial powder was weighed and then the weight equivalent to 100mg of Ceftazidime and 12.5 mg of Tazobactum was transferred into a 100 ml volumetric flask, 70ml 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 10 ml volumetric flask and made up to 10ml with diluent.

Selection of wavelength: (λ_{max})

The standard solution of mixed solution of Ceftazidime pentahydrate & Tazobactam sodium in acetonitrile was scanned by using Photo diode array detector (PDA). Wavelength 224 nm was selected for analysis in which combined drug solution showed higher absorbance.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT

Chromatographic conditions

Chromatographic separation was carried out on Inertsil ODS 250mm x 4.6 mm, 5µ. using mobile phase Ortho phosphoric acid : Acetonitrile (53:47v/v) at a flow rate 1 ml/min. Detection of both the eluents was carried out by PDA Detector [14,15] (Figure 1)

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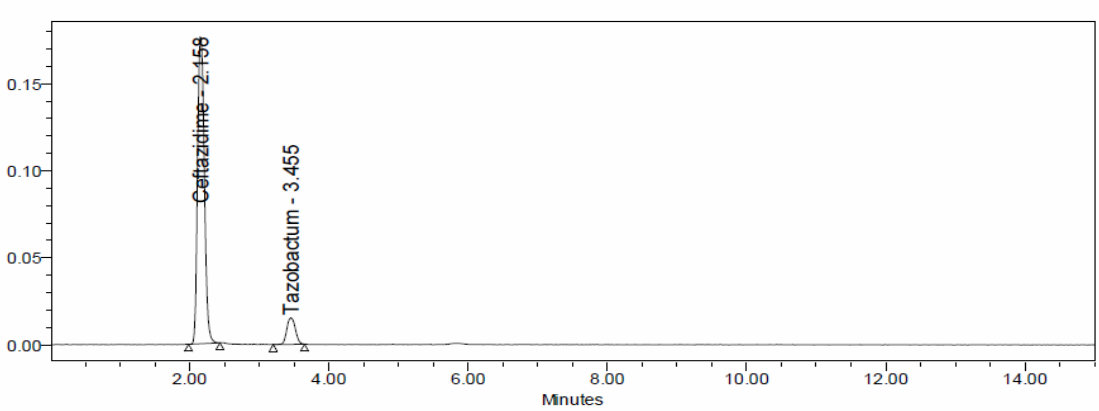

Figure 1: Chromatogram of Ceftazidime pentahydrate and Tazobactam sodium

ASSAY:

To carry out the assay of the method, %purity was calculated. Three injections of standard and three injections of sample (formulation) were injected simultaneously. The peak area were recorded and %purity was calculated (table 1, 2).

Table 1: Assay data of sample solution injections 1, 2 and 3

Peak	Name	Ret. Time	Area	Mean
1	Ceftazidime pentahydrate	2.288	705266	707990
		2.284	716028	
		2.285	702678	
2	Tazobactam sodium	3.629	55953	56190
		3.630	56892	
		3.631	55725	

Table 2: % Assay data of Ceftazidime pentahydrate and Tazobactam sodium

Name	Std. Avg. Area	Sample Avg. Area	% Assay
Ceftazidime pentahydrate	705603	707990	99.65%
Tazobactam sodium	56516	56190	98.75%

VALIDATION [13]**:**

SPECIFICITY:

The specificity of the method was ascertained by analyzing standard drug and sample (dosage form). The specificity of the method was confirmed by comparing the peaks of the sample and standard injected having the same concentration of drugs (Figure 2,3),(table 3)

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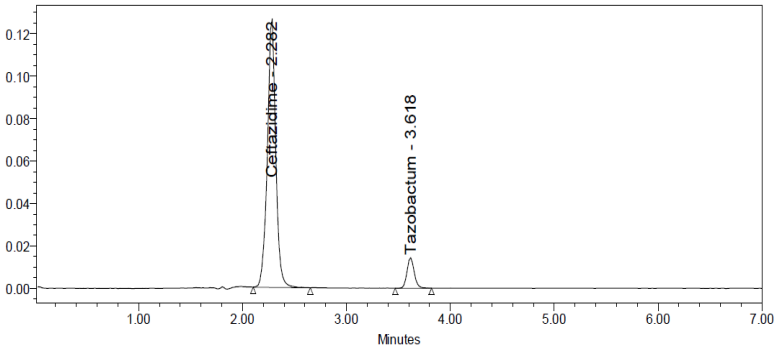

Figure 2: Chromatogram of sample injection

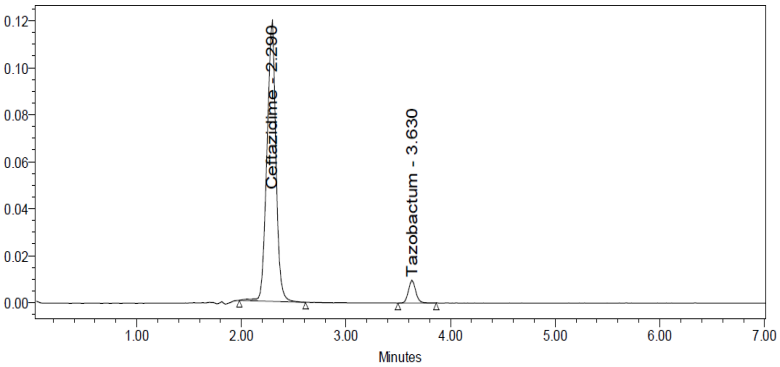

Figure 3: Chromatogram of Standard injection

Table 3: Specificity Data of Standard and sample injections

Standard Injection	Retention time	Area	Theoretical Plates	Retention time	Area	Theoretical Plates
	2.290	710709	3217	3.630	56326	12853
Sample Injection	2.282	705799	4165	3.618	5559	4165

LINEARITY:

Appropriate volumes were injected from standard stock solution containing 25-150 µg/ml of Ceftazidime pentahydrate and 3.125-18.75µg/ml of Tazobactam sodium respectively. Each standard was analyzed in six replicates and peak area was noted (Figure 4 a,b,c,d,e,f). The relationship between peak area and concentration was established by the simple regression equation method (Figure 5, 6).

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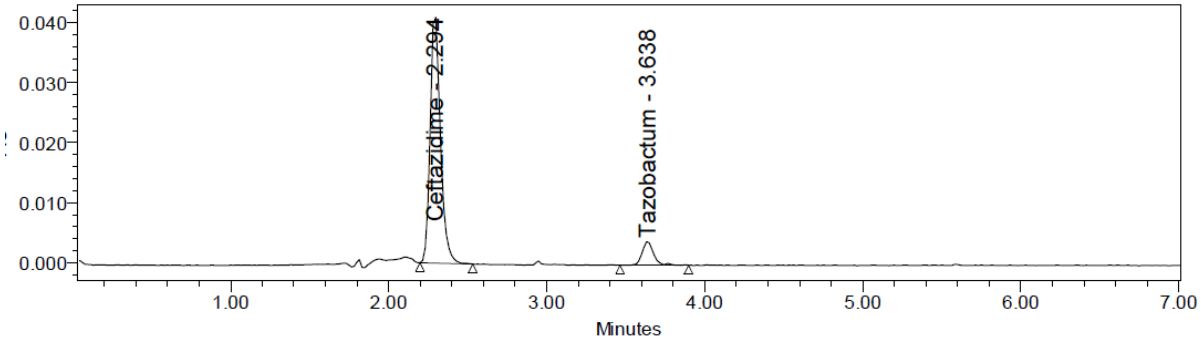

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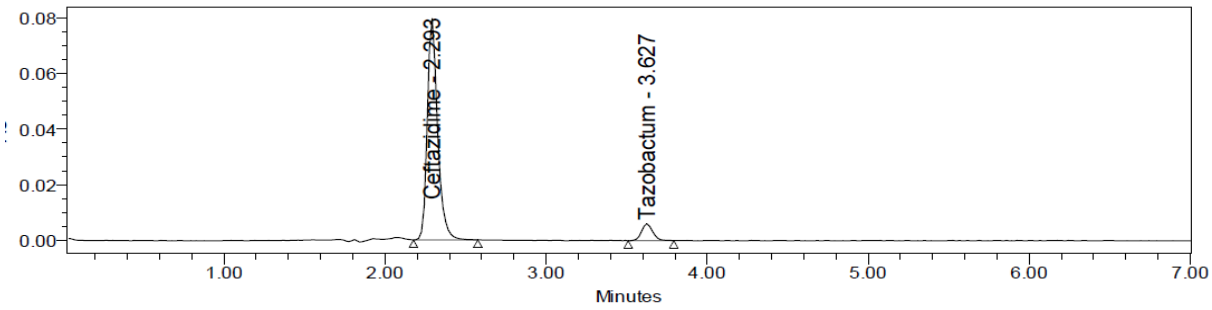

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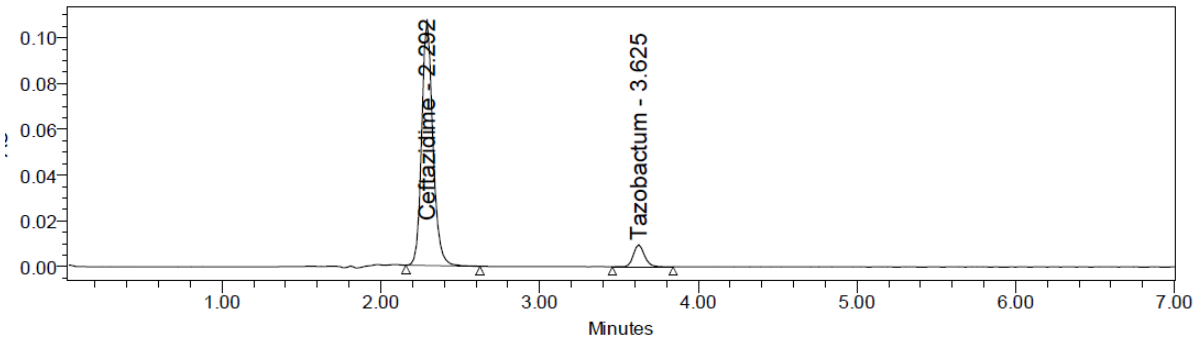

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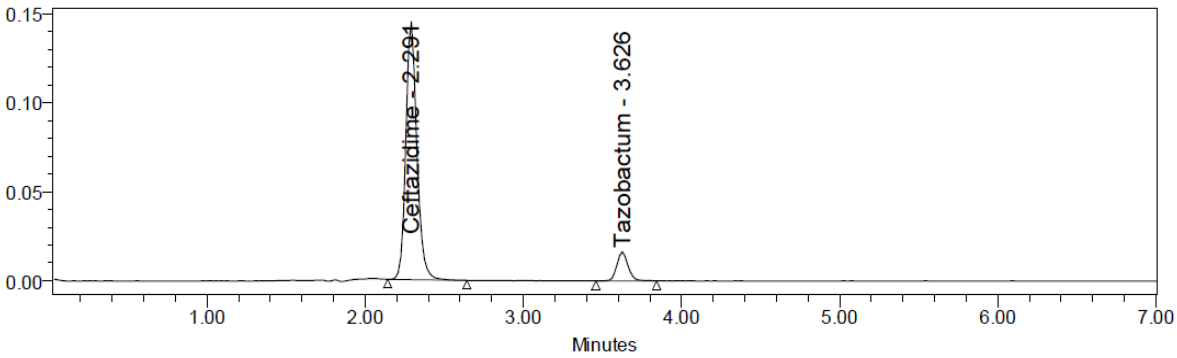

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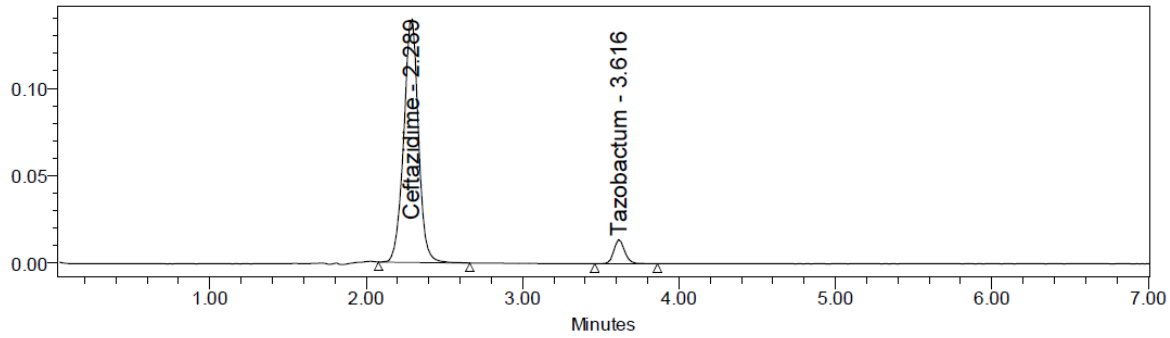

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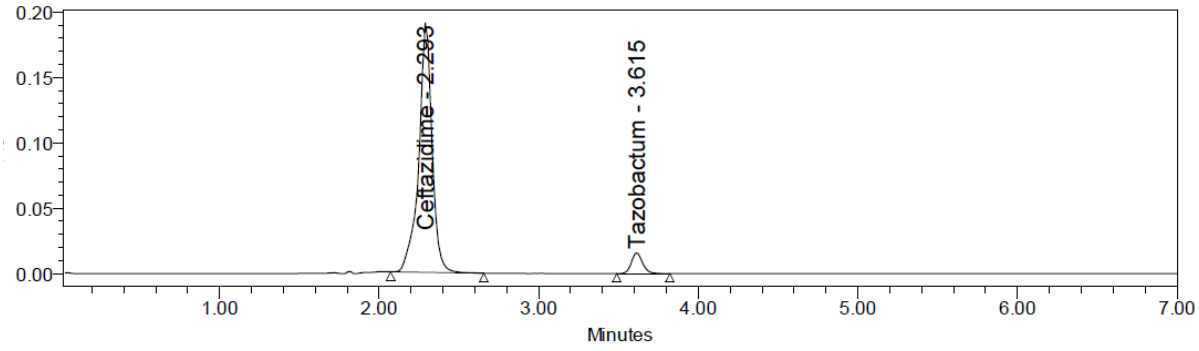

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Figure 4: (a) (b) (c) (d) (e) (f)–Chromatograms of Linearity

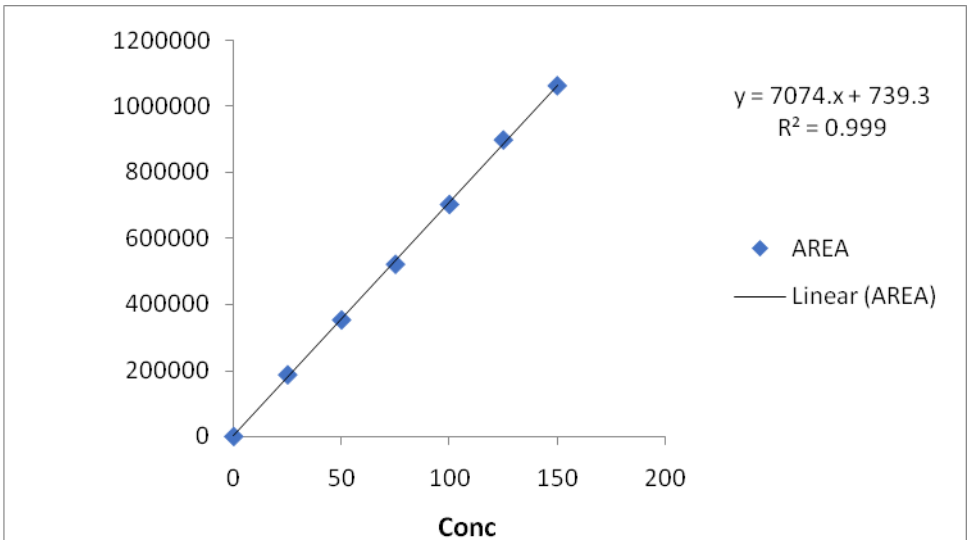

Figure 5: Calibration curve of Ceftazidime pentahydrate

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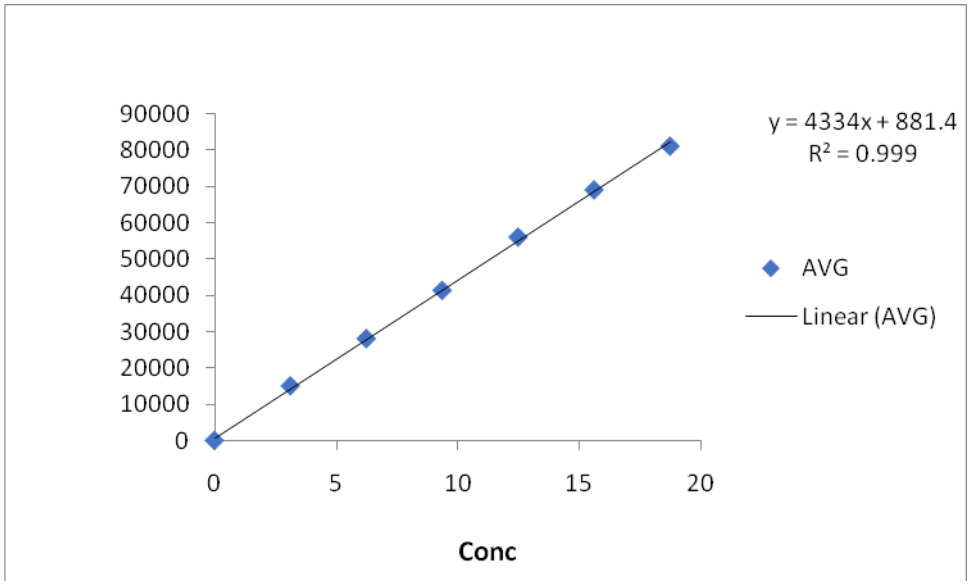
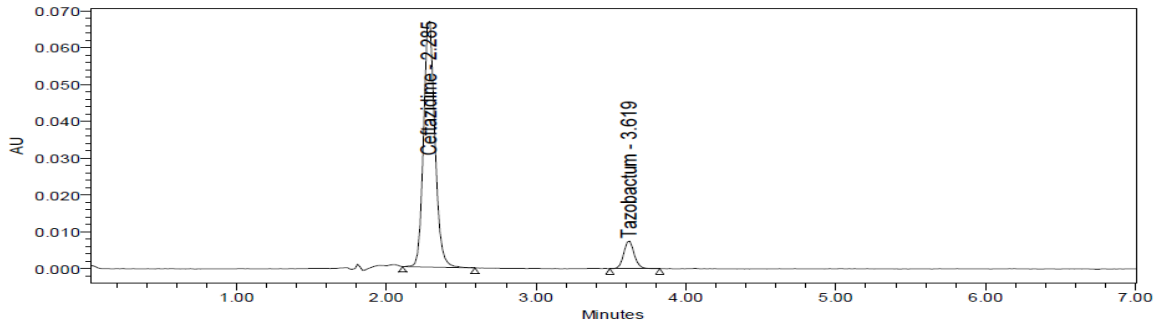


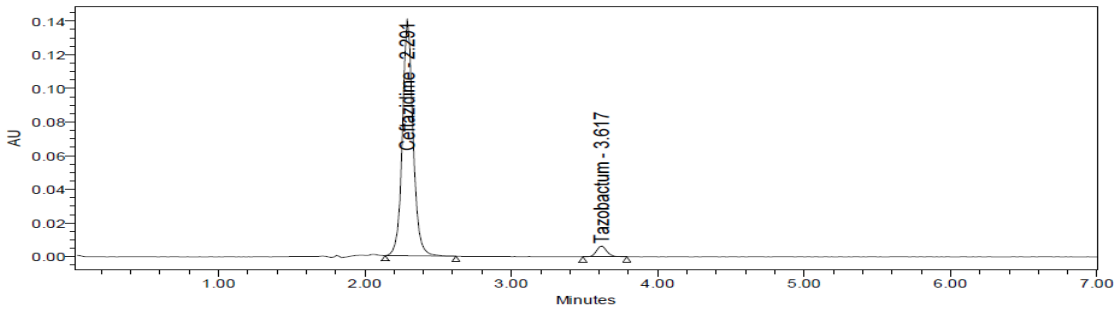
Figure 6: Calibration Curve of Tazobactam sodium

ACCURACY:

To check the accuracy in the method and recovery studies were carried out at three different levels 50%, 100%, 150%, and showed in (Figure 7 and table 4)



50%



100%

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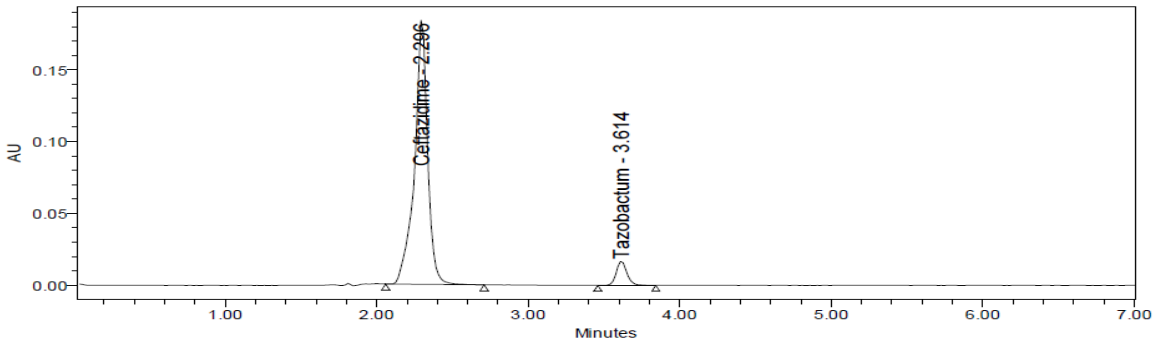

150%

Figure 7: 50%, 100%, 150% Chromatograms of Accuracy

Table 4: Accuracy data for Ceftazidime Pentahydrate and Tazobactam sodium

Conc	Amount of sample		Amount found		% Recovery	
	Ceftazidime pentahydrate	Tazobactum sodium	Ceftazidime pentahydrate	Tazobactum sodium	Ceftazidime pentahydrate	Tazobactum sodium
50	50	6.25	49.5	6.24	99.09	99.84
100	100	12.5	99.8	12.3	99.98	98.6
150	150	18.75	149.7	18.5	99.54	98.6

PRECISION:

Method Precision:

Six different sample solutions shall be prepared from the homogeneous sample and shall be analyzed using the proposed method over a short period of time by same analyst, on same equipment, on same day. The assay results and relative standard deviation of the results was calculated and given in (table 5).

Table 5: Results for Method precision of Ceftazidime pentahydrate and Tazobactam sodium

Parameters	Ceftazidime Pentahydrate	Tazobactam sodium
Avg. Retention	2.285	3.022
Standard Deviation	0.935	104.1
% RSD	0.92	0.93

ROBUSTNESS:

The robustness of the method was checked by changing flow rate, Mobile phase organic phase composition ratio and temperature and found that the plate count and tailing factor were within the limit, hence the method is robust. The data showed in (table 6)

Table 6: Robustness data of Ceftazidime and Tazobactam

	Tailing Factor	
	Ceftazidime	**Tazobactam**

Flow rate Minus	1.075	1.14
Flow rate Plus	1.074	1.13
Mobile phase Minus	1.04	1.16
Mobile phase Plus	1.075	1.135
Temperature Minus	1.025	1.115
Temperature Plus	1.02	1.13

Limit of Detection and Limit of Quantification (LOD and LOQ)

The LOD and LOQ were estimated from the calibration curve. The LOD may be calculated as

$$LOD = 3.3 \times (SD/Slope)$$

The LOQ may be calculated as

$$LOQ = 10 \times (SD/Slope)$$

Where, SD = Standard deviation of the Y-intercept of the calibration curve.

Slope = Mean slope of the calibration curve.
LOD and LOQ of Ceftazidime pentahydrate was found to be 0.34 and 1.04.
LOD and LOQ of Tazobactam sodium was found to be0.67 and 2.0.
LOD and LOQ Calculated from the linearity calibration curves.

DISCUSSION

The RP-HPLC method was developed and validated as per ICH guidelines for the estimation of Ceftazidime pentahydrate and Tazobactam sodium. The simultaneous estimation ofCeftazidime pentahydrate and Tazobactam sodium were carried out by HPLC using Ortho Phosphoric Acidand Acetonitrile as mobile phase and Intersil ODS 250mm x 4.6 mm, 5µ.column as a stationary phase and selected for the wavelength at 224 nm for estimation of Ceftazidime pentahydrate and Tazobactam sodium. The method wasvalidated for precision, accuracy, linearity, system suitability, LOD and LOQand robustness studies. The system suitability parameter reveals that the values withinthe specified limit for proposed method. % RSD of Ceftazidime pentahydrate and Tazobactam sodium was not more than2.0 and the tailing factor was not more than 1.5. The precision of the system andmethod were checked and found to be within limits. It indicates that the method isprecise. The linearity study covers the range from 25 – 150 ppm for ceftazidime pentahydrate and 3.125 – 18.75 ppm for Tazobactam sodium with respect to testconcentration and got the correlation coefficient 0.999 and 0.999. The result reveals that themethod is linear at specification level. From the results shown in the accuracy table, itwas found that recovery value of pure drugs were99.6 % and 99.10 %for Ceftazidime pentahydrate and Tazobactam sodium, which indicates the methodis accurate and excipients and additives were well separated from the Ceftazidime pentahydrate and Tazobactam sodium. The data was shown in the table 5. So that the proposed method was accurate. The robustness ofthe method checked by changing flow rate, mobile phase, temperature and found that systemsuitability parameters were within the limit, hence the method is robust.The methodcan be used for the estimation of Ceftazidime pentahydrate and Tazobactam sodium in their dosageform and this method is Linear, Accurate, and Precise.

Table 7: Summary of the validation parameters of proposed method

Parameters	**Ceftazidime**	**Tazobactum**
Accuracy/trueness	99.6 %	99.10 %
System Precision	0.64	0.8
Method Precision	0.93	2.1

DetectionLimit	0.34	0.67
QuantizationLimit	1.04	2.0
Linearity	25-150 ppm	3.125-18.75 ppm

CONCLUSION

A simple and sensitive stability indicating RP-HPLC method was developed for simultaneous estimation of Ceftazidime pentahydrate and Tazobactam sodium. It concludes that all the parameters are within the limits and meet the acceptance criteria of ICH guidelines for method validation. The proposed method was simple, accurate, specific, precise, robust and economical. Hence this method is validated and can be used for routine and stability sample analysis.

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