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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:
Objective: *A simple, Accurate, precise method was developed for the simultaneous estimation of the Ceftolozane and Tazobactam in pharmaceutical dosage form.*
Methods: *Chromatogram was run through XTerra C18 (4.6 x 150mm, 5 µm particle size). Mobile phase containing Phosphate buffer and Acetonitril in the ratio of 55:45 was pumped through column at a flow rate of 1ml/min. Buffer used at pH 4.6. Temperature was maintained at Ambient. Optimized wavelength for Ceftolozane and Tazobactam was 260 nm.*
Results: *Retention time of Ceftolozane and Tazobactam were found to be 2.28 min and 3.62 min. The % purity of Ceftolozane and Tazobactam was found to be 100.5% and 101.2% respectively. The system suitability parameters for Ceftolozane and Tazobactam such as theoretical plates and tailing factor were found to be 2589.3, 5419.7, 1.11 and 1.34. The resolution was found to be 8.0. The linearity study for Ceftolozane and Tazobactam correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 100.1% and 100.4%, %RSD for repeatability was 1.2 and 0.60, % RSD for intermediate precision was 1.48 and 0.82 respectively. The precision study was precise, robust and repeatable. LOD value was 0.63 and 0.09, and LOQ value was 1.88 and 0.26respectively.*
Conclusion: *The results of study showed that the proposed RP-HPLC method is a simple, accurate, precise, rugged, robust, fast and reproducible, which may be useful for the routine estimation of Ceftolozane and Tazobactam in pharmaceutical dosage form.*
Keywords: *Ceftolozane, Tazobactam, RP-HPLC, Simultaneous estimation.*

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INTRODUCTION:

Ceftolozane is a cephalosporin antibiotic used to treat complicated intra-abdominal infections in combination with metronidazole, complicated urinary tract infections, and hospital-acquired pneumonia.[1] Ceftolozane belongs to the cephalosporin class of antibacterial drugs. Ceftolozane exerts antibacterial effects, preventing the formation of cell walls that protect bacteria from injury and confer resistance to some antibiotics. Its antibacterial activity is also mediated through ceftolozane binding to penicillin-binding proteins (PBPs), which are required for peptidoglycan cross-linking for bacterial cell wall synthesis. As a result of cell wall synthesis inhibition, bacterial cells are killed, treating various infections.[2] IUPAC Name is 5-amino-2-{[(6R,7R)-7-[(2Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-[(1-carboxy-1-methylethoxy) imino] acetamido]-2-carboxylato-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-en-3-yl] methyl}-4-{[(2-aminoethyl) carbamoyl] amino}-1-methyl-1H-pyrazol-2-ium. Chemical Formula is $C_{23}H_{30}N_{12}O_8S_2$. Molecular weight is 666.69. Soluble in water (50mg/ml); slightly soluble in methanol and 100% ethanol; insoluble in acetone, chloroform and benzene.

Tazobactam is a beta lactamase inhibitor administered with antibiotics such as piperacillin and ceftolozane to prevent their degradation, resulting in increased efficacy.[3] Tazobactam broadens the spectrum of piperacillin and ceftolozan by making them effective against organisms that express beta-lactamase and would normally degrade them. This occurs through the irreversible inhibition of beta-lactamase enzymes. In addition, tazobactam may bind covalently to plasmid-mediated and chromosome-mediated beta-lactamase enzymes. Tazobactam is predominantly effective against the OHIO-1, SHV-1, and TEM groups of beta-lactamases, but may also inhibit other beta-lactamases.[4] IUPAC Name is (2S,3S,5R)-3-methyl-4,4,7-trioxo-3-(1H-1,2,3-triazol-1-ylmethyl)-4λ⁶-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid. Chemical Formula is C10H12N4O5S. Molecular weight is 300.29. Tazobactam is slightly soluble in aqueous solution (9.59 mg/mL). Tazobactam sodium is freely soluble in aqueous solution.

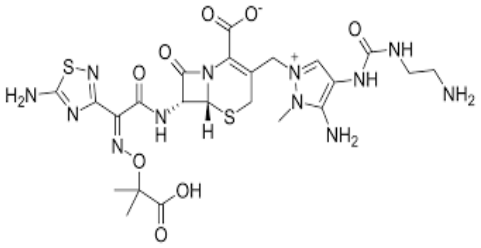

Figure 1: Structure of Ceftolozane

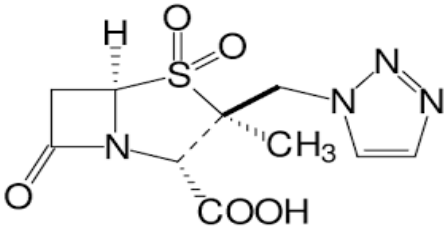

Figure 2: Structure of Tazobactam

The literature survey revealed that There are Various analytical methods were carried out for the estimation of Ceftolozane and Tazobactum as a single or combined with other drugs in pharmaceutical dosages Literature survey reveals that the retention time for the simultaneous estimation of Ceftolozane and Tazobactum is more. Hence the present study, we had made an attempt to develop simple, accurate, precise, less time consuming and with less retention time using RP-HPLC for the simultaneous estimation of Ceftolozane and Tazobactum in bulk and pharmaceutical dosage form by RP-HPLC.[5-11] To validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form.

MATERIALS AND METHODS:

Chemicals and Reagents: Ceftolozane and Tazobactum were Purchased from market. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was

carried out at 260 nm with column XTerra C18 (4.6 x 150mm, 5 µm particle size), dimensions at 25^0C temperature. The optimized mobile phase consists of Phosphate buffer and Acetonitril in the ratio of 55:45. Flow rate was maintained at 1 ml/min.

Preparation of solutions:

Preparation of 0.1%OPAbuffer:

0.1ml of ortho phosphoric acid was taken in a 1000ml volumetric flask and solution was filtered by using 0.45-micron membrane filter and sonicated for 10 min.

Preparation of mobile phase:

550 ml (55%) of OPA buffer and 450 ml of Acetonitrile (45%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent: Mobile phase was used as diluent

Preparation of stock standard solutions:

Accurately weighed and transfer25mg&12.5mg of Ceftolozane and Tazobactam working standards into a 25ml clean dry volumetric flask respectively, sonicated for 30 minutes and make up to the final volume with diluents. The above standard stock solution suitably diluted with diluents to obtain various concentrations of Ceftolozane and Tazobactam.

Preparation of working standard solutions: Working standard solutions were prepared by taking 1ml of stock solutions of Ceftolozane and Tazobactam in to clean dry 10ml volumetric flask and make up volume with diluent to get a concentration of 100µg/ml of Ceftolozane and 50µg/ml Tazobactam.

Preparation of Sample Solutions of Ceftolozane and Tazobactam:

One vial powder was weighed and powder equivalent to 850 mg of ceftolozane and tazobactam was taken into 100 ml clean dry volumetric flask, diluent was added and sonicated to dissolve completely and volume was made up with the diluent. The above sample solution was filtered, 1ml of filtrate was pipette out into a 10 ml volumetric flask and made up to 10ml with diluent

Procedure:

20µL of the standard, sample are injected into the chromatographic system and the areas for ceftolozane and tazobactam peaks are measured and the %Assay are calculated by using the formulae.

METHOD:

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into XTerra C18 (4.6 x 150mm, 5 µm particle size), the mobile phase of composition Phosphate buffer and Acetonitril in the ratio of 55:45 was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine ceftolozane and tazobactam in their tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method:

Linearity: The linearity study was performed for the concentration of 25 ppm to 150 ppm and 12.5 ppm to 75ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. 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. The resulte are shown in table 3.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150% and 25%, 50%, 75% Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for ceftolozane and tazobactam and calculate the individual recovery and mean recovery values. The results are shown in table 4,5.

Precision Studies: precision was caliculated from Coefficient of variance for six replicate injections of

the standard. 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. The resulte are shown in table 6.

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found. The resulte are shown in table 7,8.

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. The flow rate was varied at 0.8 ml/min to 1.2 ml/min. The resulte are shown in table 9,10,11.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The resulte are shown in table 12.
LOD = 3.3σ/S and
LOQ = 10 σ/S, where
σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

RESULTS AND DISCUSSION:

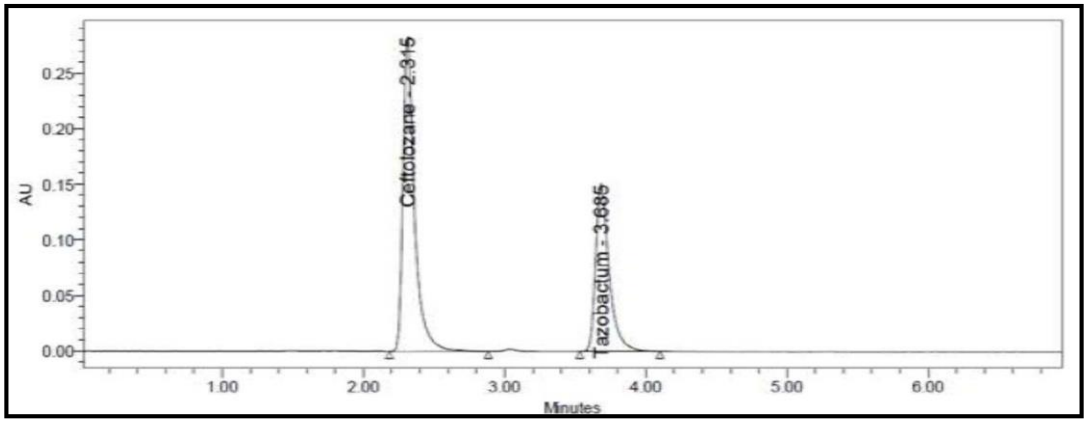

Figure 3: Standard chromatogram

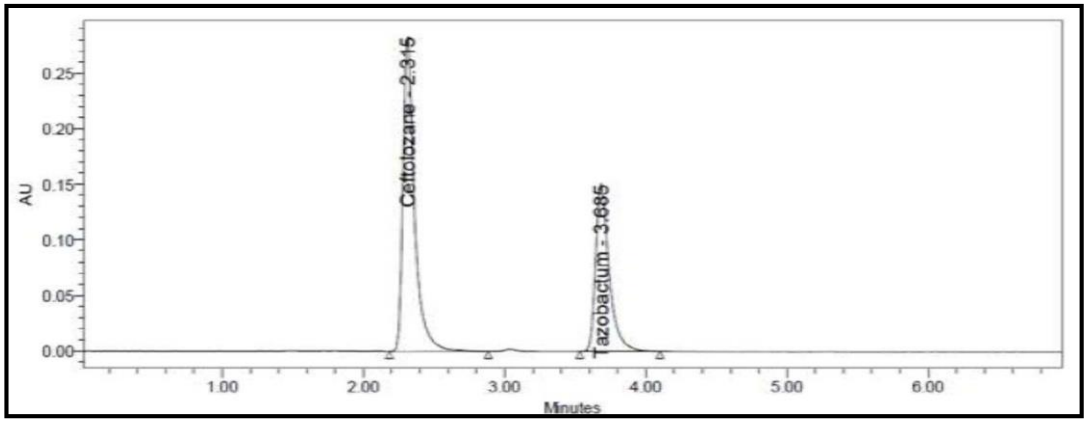

Figure 4: Sample chromatogram

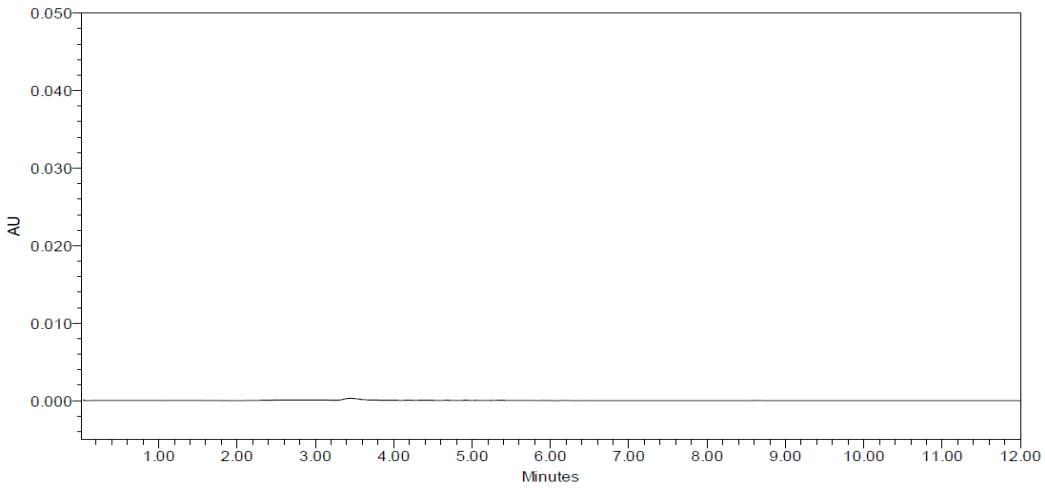

Figure 5: Blank chromatogram

Table 1: System suitability parameters

Parameter	Ceftolazone	Tazobactam
Peak area	924081	22127
Theoretical plates	2589.3	5419.72
Retention time	2.28	3.62
Tailing factor	1.11	1.34

Table 2: Assay results for Ceftolazone and Tazobactam

	Label Claim (mg)	% Assay
Ceftolazone	25	100.3
Tazobactam	12.5	101.6

Table 3: Linearity results of Ceftolazone and Tazobactam

Level	Concentration of Ceftolazone (µg/ml)	Peak area	Concentration of Tazobactam (µg/ml)	Peak area
1	25	365031	12.5	7362
2	50	590445	25	14723
3	75	824680	37.5	22084
4	100	938891	50	29512
5	125	1262631	62.5	36368
6	150	1482624	75	44237

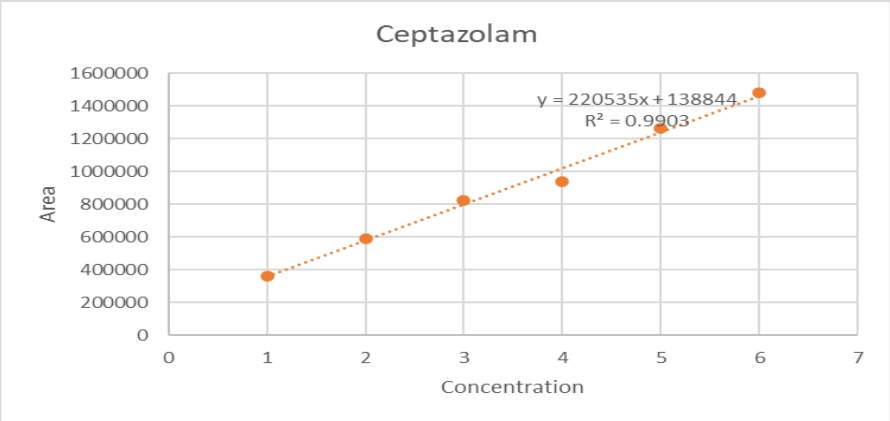

Figure 6: Linearity graph for Ceftolozane

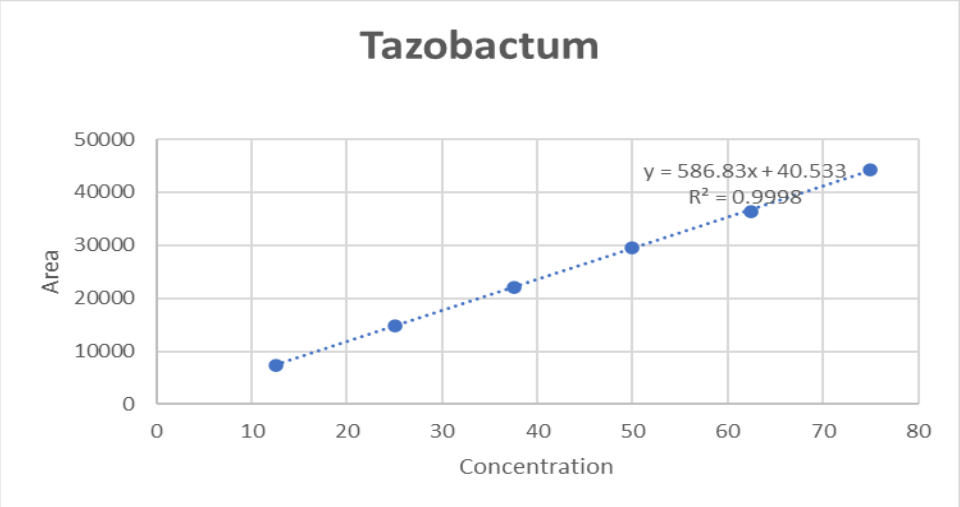

Figure 7: Linearity graph for Tazobactam

Table 4: Showing accuracy results for Ceftolazone

Sample name	Amount added (µg/ml)	Amount found (µg/ml)	%Recovery	Statistical Analysis
S1:50%	50	49.8	99.6	Mean=100.12%(n=3)
S2:50%	50	49.6	99.2	S.D=1.031
S3:50%	50	50.78	101.56	%RSD=1.030
S4:100%	100	100.56	100.56	Mean=100.53%(n=3)
S5:100%	100	100.45	100.45	S.D=0.060
S6:100%	100	100.59	100.59	%RSD=0.060
S7:150%	150	150.55	100.36	Mean=99.59%(n=3)
S8:150%	150	148.36	98.90	S.D=0.598
S9 :150%	150	149.29	99.52	%RSD=0.601

Table 5: Showing accuracy results for Tazobactam

Sample name	Amount added (µg/ml)	Amount found (µg/ml)	%Recovery	Statistical Analysis
S1:50%	25	25.65	102.6	Mean=101.11%(n=3)
S2:50%	25	24.77	99.08	S.D=1.48
S3:50%	25	25.41	101.64	%RSD=1.46
S4:100%	50	50.18	100.36	Mean=100.43%(n=3)
S5:100%	50	49.71	99.42	S.D=0.85
S6:100%	50	50.76	101.52	%RSD=0.85
S7:150%	75	76.25	101.66	Mean=100.40%(n=3)
S8:150%	75	75.44	100.58	S.D=1.11
S9 :150%	75	74.22	98.96	%RSD=1.10

Table 6: Precision results for Ceftolazone and Tazobactam

Ceftolazone				Tazobactam		
S. No	Concentration (µg/ml)	Peak Area	% Assay	Concentration (µg/ml)	Peak Area	% Assay
1	100	911508	99.3	50	22376	98.4
2	100	939016	100.2	50	21765	101.45
3	100	908096	100.4	50	21597	99.38
4	100	940019	99.4	50	21572	101.92
5	100	924217	100.9	50	21733	100.9
6	100	921693	99.6	50	22476	99.6
Average		924091.5	99.97		21919.8	100.28
SD		13389.5	0.63		400.4	1.36
%RSD		1.4	0.63		1.8	1.36

Table 7. Ruggedness results of Ceftolozane

Laboratory-1 (% Assay)-HPLC-1					Laboratory-2 (% Assay)-HPLC-2			
	Analyst-1		Analyst-2		Analyst-1		Analyst-2	
Concentration (µg/ml)	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2
100	99.45	97.25	98.25	99.47	102.08	101.08	102.38	101.51
100	98.50	99.27	101.27	100.30	101.87	100.26	100.18	100.18
100	97.09	96.91	99.22	99.19	99.38	100.71	101.61	100.51
100	99.48	98.18	99.40	98.42	101.90	99.78	100.39	101.81
100	99.34	100.13	97.08	99.28	100.20	99.23	101.82	101.47
100	100.24	98.09	100.24	101.08	100.29	100.78	101.27	101.29
Average	99.02	98.31	99.24	99.62	100.95	100.31	101.28	101.13
SD	1.09	1.22	1.47	0.93	1.14	0.70	0.85	0.64
%RSD	1.10	1.24	1.48	0.94	1.13	0.69	0.84	0.63

Table 8. Ruggedness results of Tazobactam

Laboratory-1 (% Assay)-HPLC-1					Laboratory-2 (% Assay)-HPLC-2			
	Analyst-1		Analyst-2		Analyst-1		Analyst-2	
Concentration (µg/ml)	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2
100	101.83	102.54	100.21	101.34	100.21	98.37	100.21	98.35
100	100.18	101.29	99.81	100.65	100.61	101.67	99.47	100.52
100	100.38	100.51	101.3	99.78	99.4	98.02	101.82	102.78
100	100.39	101.81	100.61	101.81	100.39	98.42	100.74	99.58
100	100.65	101.72	100.8	101.72	97.56	99.28	101.47	102.55
100	101.27	101.29	99.79	100.27	101.27	100.69	102.27	99.79
Average	101	101.53	100	100.93	100	99	101	101
SD	0.6	0.68	0.6	0.82	1.3	1.5	1.1	1.8
%RSD	0.6	0.67	0.6	0.82	1.3	1.5	1.0	1.7

Robustness results

Table 9: Flow variation results for Ceftolozane and Tazobactam

Drug	Change in Flowrate (ml/min)	Change in flow Rate (0.8ml/min to 1.2 ml/min)		
		%Assay	SD	% RSD
Ceftolazone	0.8	98.2	1.2	1.34
	1	101.41	1.14	1.2
	1.2	99.26	1.6	1.64
Tazobactam	0.8	100.12	1.7	1.8
	1	98.46	0.79	0.8
	1.2	101.12	1.43	1.5

Table 10: Change in Mobile phase composition results for Ceftolozane and Tazobactam

Drug	Change in mobile phase	n Mobile phase (0.8ml/min to 1.2 ml/min)		
		%Assay	SD	% RSD
Ceftolazone	10% less organic phase	101.21	0.95	1.1
	Actual	99.42	1.28	1.3
	10% more organic phase	100.61	1.26	1.3
Tazobactam	10% less organic phase	100.81	1.43	1.5
	Actual	101.21	0.58	0.6
	10%more organic phase	99.41	1.4	1.5

Table 11: Change in column Temparature for Ceftolazone and Tazobactam

Drug	ange in column temperature	Change in column temperature		
		%Assay	SD	% RSD
Ceftolazone	25°C	98.34	1.56	1.6
	30°C	101.42	1.26	1.3
	35°C	101.39	1.40	1.5
Tazobactam	25°C	101.45	1.58	1.6
	30°C	99.45	0.49	0.5
	35°C	99.81	0.61	0.7

Table 12: LOD, LOQ of Ceftolozane and Sitagliptin

Drug	**LOD**	**LOQ**
Ceftolozane	0.63	1.88
Sitagliptin	0.09	0.27

CONCLUSION:

The Developed HPLC method was validated and it was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Ceftolozane and Tazobactam in its pure form and in its pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Ceftolozane and Tazobactam in pure and its pharmaceutical dosage forms.

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