

## Method Development and Validation for the Simultaneous Estimation of Ceftriaxone and Tazobactam in Bulk and Pharmaceutical Dosage Forms by Rp-Hplc

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Date of Submission: 15-09-2025

Date of Acceptance: 25-09-2025

### ABSTRACT

A simple, accurate and precise High Performance Liquid Chromatographic (HPLC) method was developed and validated for the simultaneous estimation of Ceftriaxone and Tazobactam in bulk and Pharmaceutical dosage forms. The method was developed using Waters HPLC system with Inertsil – C18, ODS column (250 x 4.6 mm, 5 $\mu$ ). Mobile phase containing Methanol and buffer in the ratio 80:20 v/v in isocratic elution mode at a flow rate of 1.0ml/min (load of 20 $\mu$ l). The detection was carried out at 254 nm. Retention time of Ceftriaxone and Tazobactam were found to be 3.049 min and 4.317 min respectively. The developed method was validated with respect to linearity, robustness, precision and accuracy. It was successfully applied for the simultaneous quantitative estimation of Ceftriaxone and Tazobactam in the Pharmaceutical dosage form. Results show that the retention and run time were decreased, so it is evident that the method developed was simple and economical that can be adopted in regular Quality control test in Industries

**Key words:** HPLC, Ceftriaxone, Tazobactam, Simultaneous estimation, Method validation, Inertsil C18, Quality control.

### I. INTRODUCTION

Ceftriaxone belongs to the class cephalosporin antibiotic. ceftriaxone is used to treat bacterial infections mostly gram-positive, organisms. Ceftriaxone works by preventing the bacterial cell wall from synthesizing mucopeptides. The beta-lactam molecule of Ceftriaxone binds to carboxypeptidases, endopeptidases, and transpeptidases in the bacterial cytoplasmic membrane. These enzymes are necessary for cell division and cell wall production. By attaching to

these enzymes, ceftriaxone causes the production of defective cell walls and cell death.(10)

Tazobactam belongs to the category of beta-lactamase inhibitors. It is combined with Piperacillin and Ceftolozane for the treatment of variety of bacterial infections. Tazobactam broadens the spectrum of piperacillin and ceftolozane by making them effective against organisms. This occurs through irreversible inhibition of beta-lactamase enzymes. (11)

### II. MATERIALS AND METHODS

- **Materials:** Ceftriaxone, Tazobactam, combination of Ceftriaxone and Tazobactam formulation, HPLC grade Methanol, acetonitrile, water.
- **Instrument:** HPLC instrument used was of WATERS HPLC SYSTEM 2690/5 with Auto Injector and PDA Detector. Software used is Empower 2. UVVIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Ceftriaxone and Tazobactam solutions.(1-9)

- **Preparation of solutions: (1-2)**

**Preparation of a stock solution:** 100 mg of Ceftriaxone and Tazobactam each were weighed accurately and dissolved separately in 100 ml volumetric flasks, sonicated for 20 min to obtain 1000g/ml of Ceftriaxone and Tazobactam respectively.

**Preparation of working standard solution:** From the above standard stock solution 4 ml from each solution was taken into a 100 ml volumetric flask then made up the volume with diluents and sonicated for 10 min and filtered through 0.45 $\mu$ m membrane filter.

### III. RESULTS AND DISCUSSION

**Method Validation:** Validation parameters includes specificity, linearity, range, accuracy, precision, repeatability, intermediate precision, limit of detection, limit of quantification, robustness.(12-15)

**Specificity:** Specificity is the ability to assessing equivocally the analyte in the presence of components which may be expected to be present. Typically these components include impurities, degradants, matrix etc. Blank solution and standard solutions of Ceftriaxone (40µg/ml) and Tazobactam (40µg/ ml) were injected into the HPLC system. The peak purity data of Ceftriaxone and Tazobactam were compared. There should not be any interference at the retention time of the main peaks.

**Linearity:** Linearity for the drugs Ceftriaxone and Tazobactam was determined by preparing the standard solutions at seven concentrations levels in the range of 20-80µg/ml for Ceftriaxone and 20-80µg/ml for Tazobactam from stock solution. The linearity charts of Ceftriaxone and Tazobactam was shown in the figure no 2&3. The correlation coefficient was found to be 0.9993 and 0.9997 for Ceftriaxone and Tazobactam respectively. Linearity results were tabulated in table 2. (16-18)

**Accuracy:** Accuracy was performed by spiking known amounts of standard solution to sample solution at three different concentrations levels (50%, 100%, 150%) and there by analyzed for %RSD which should not be more than 2.0.The %

recovery was calculated and the results was reported in table no. 3 & 4.

**Precision:** The precision of the analytical method was studied by injecting six replicates of standard containing 40µg/ml of Ceftriaxone and 40µg/ml of Tazobactam which were injected into HPLC system. The % RSD was calculated and the results were reported in the table no.5 & 6.

**Limit of Detection (LOD) and Limit of Quantification (LOQ): (19-22)**

The limit of detection was defined as the concentration which yields a signal - to - noise ratio 3:1 where as the limit of quantification was calculated to be the lowest concentration that could be measured with signal - to - noise ratio10:1. LOD and LOQ were calculated from slope and standard deviation. The results were tabulated in table no. 7.

**Robustness:** The smallest deliberate changes in method like change in flow rate are made but there were no predictable changes in the results and are in the range as per ICH guidelines. Conditions like decrease flow rate (0.8 ml/min), increases flow rate (1.2 ml/min) was maintained and sample were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. % RSD was found to be within the limits and results were tabulated in table no. 8.

**Assay:** Assay was conducted on marketed formulation and mean % assay was found. The results were tabulated in table no. 9.

**Table 1: Chromatographic conditions for optimized method**

Parameters	Method
Stationary phase (column)	Inertsil -ODS C <sub>18</sub> (250 x 4.6 mm, 5 µ)
Mobile Phase	Methanol : Buffer (80:20)
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	10 min
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	254nm
Drug RT (min)	3.049min for Ceftriaxone and 4.317min for Tazobactam.

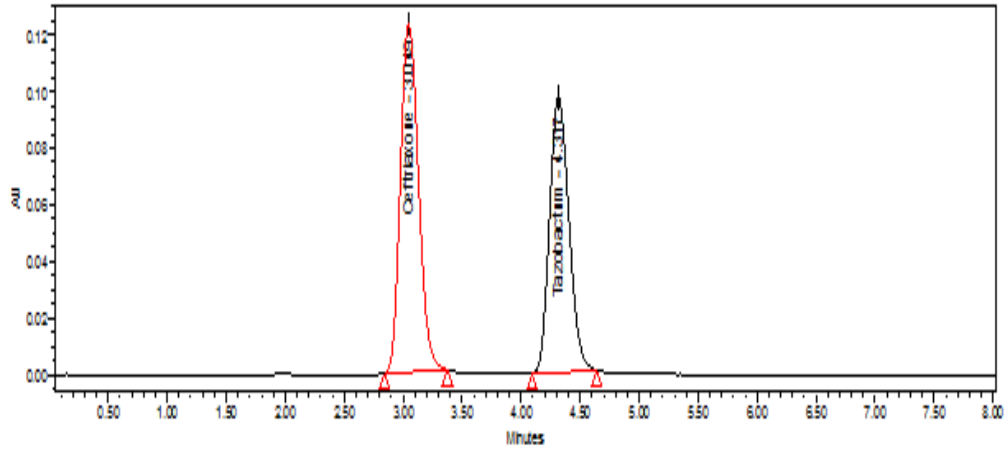
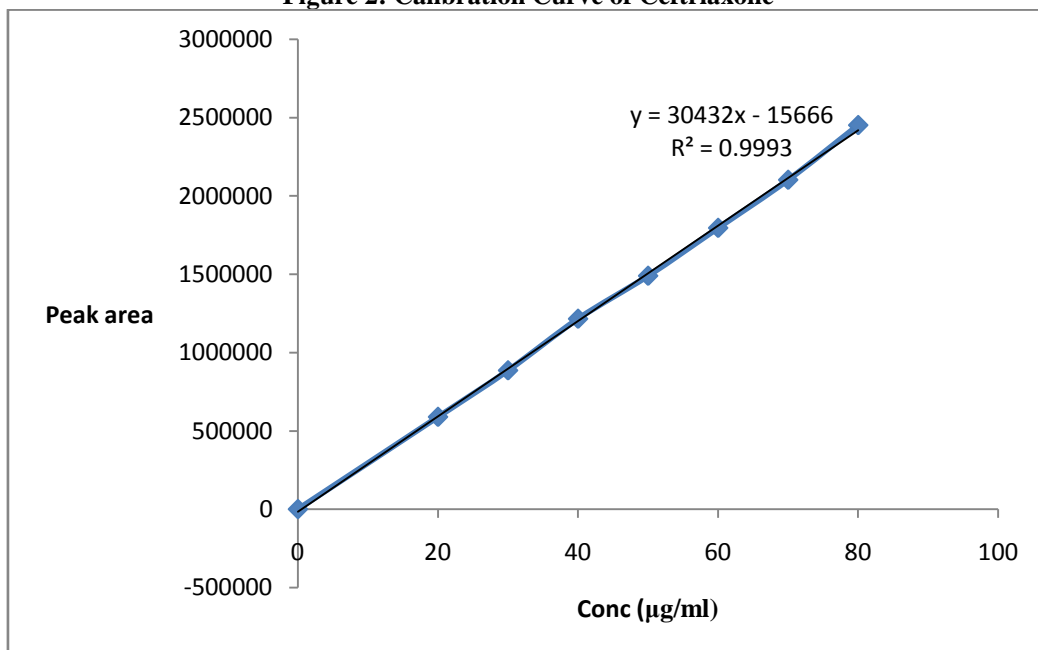


Figure 1: Optimized chromatogram

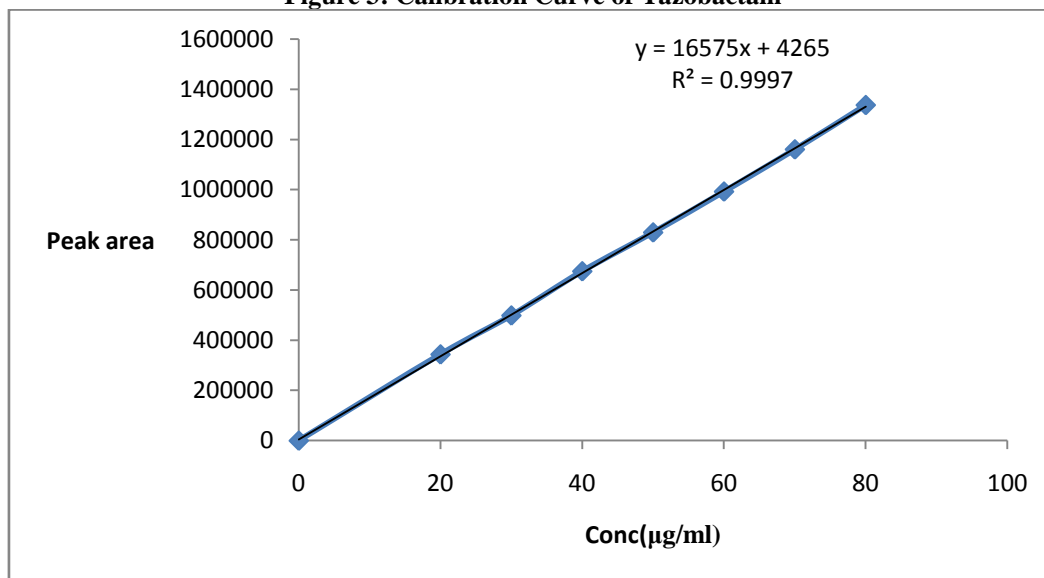
Table 2: Linearity results of Ceftriaxone and Tazobactam

Ceftriaxone		Tazobactam	
Conc (µg/ml)	Peak area	Conc(µg/ml)	Peak area
20	588735	20	343650
30	885434	30	498630
40	1214943	40	674665
50	1489197	50	829406
60	1794937	60	992122
70	2101821	70	1160122
80	2450946	80	1336708

Figure 2: Calibration Curve of Ceftriaxone



**Figure 3: Calibration Curve of Tazobactam**



**Table 3: Accuracy data of Ceftriazone**

% level	Amount spiked (µg)	Amount recovered (µg)	% Recovery	Statistical Analysis of % Recovery
50%	20	20.15	100.75	MEAN = 99.69 %RSD = 0.92
50%		19.86	99.31	
50%		19.80	99.02	
100 %	40	39.88	99.70	MEAN = 99.83 %RSD = 0.41
100 %		40.12	100.30	
100%		39.80	99.50	
150%	60	60.12	100.21	MEAN = 99.97 %RSD = 0.31
150%		59.76	99.61	
150%		60.06	100.10	

**Table 4: Accuracy data of Tazobactam**

% level	Amount spiked (µg)	Amount recovered (µg)	% Recovery	Statistical Analysis of % Recovery
50%	20	19.95	99.75	MEAN = 99.95 %RSD = 1.26
50%		20.14	100.7	
50%		19.64	98.2	
100 %	40	39.95	99.87	MEAN = 100.08 %RSD = 0.215
100 %		40.12	100.3	
100%		40.03	100.07	
150%	60	59.84	99.73	MEAN = 100.45 %RSD = 0.85
150%		60.84	101.40	
150%		60.14	100.23	

**Table 5: System Precision data of Ceftriaxone and Tazobactam**

S. No	Peak area	
	Ceftriaxone	Tazobactam
1	1239704	676488
2	1246846	683935
3	1252530	686924
4	1261073	687698
5	1266667	694665
Mean	1253364	685942
SD	10795.53	6586.819
% RSD	0.861324	0.960259

**Table 6: Method Precision data of Ceftriaxone and Tazobactam**

S. No	Peak area	
	Ceftriaxone	Tazobactam
1	1214943	674665
2	1220150	672015
3	1220212	672211
4	1219505	677612
5	1265543	689531
6	1220150	672015
Mean	1226751	676341.5
SD	19113.65	6824.749
% RSD	1.558071	1.009068

**Table 7: LOD and LOQ data of Ceftriaxone and Tazobactam**

Drug name	LOD (µg/ml)	LOQ (µg/ml)
Ceftriaxone	0.228	0.692
Tazobactam	0.251	0.759

**Table 8: Robustness data of Ceftriaxone and Tazobactam**

S. No	Drug name	Condition	Peak area	Tailing factor
1	Ceftriaxone	Flow rate(-) 0.8 ml/min	1228071	0.889
2		Flow rate(+) 1.2 ml/min	1253834	0.879
3	Tazobactam	Flow rate(-) 0.8 ml/min	677207	1.154
4		Flow rate(+) 1.2 ml/min	693574	1.149

**Table 9: Assay data Ceftriaxone and Tazobactam**

S. No	Peak area of Ceftriaxone	% Assay	Peak area of Tazobactam	% Assay
1	1214943	99.08%	674665	99.64%
2	1220150		672015	
3	1220212		672211	
4	1219505		677612	
5	1265543		689531	
6	1239704		676488	
Mean	1230010		677087	
%RSD	1.579467	0.959095		

#### IV. CONCLUSION:

The developed RP-HPLC method was validated as per ICH guidelines. All the system suitability parameters were within the range as stated by ICH guidelines. Interference peaks were not observed in blank, standard and sample chromatogram. Hence simple, precise and accurate, sensitive, specific and robust method was developed and validated. This can be used in quality control department with respect to routine analysis.

**Acknowledgements:** Authors are thankful to the management of Viswanadha Institute of Pharmaceutical Sciences (VNIPS) for providing facilities and support to carry out this work.

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