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Analytical method development and validation for the simultaneous estimation of ceftolozane and tazobactam in pure and pharmaceutical dosage form by RP-HPLC

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ABSTRACT

The purpose of the present study was to develop a simple, accurate and precise method for the simultaneous estimation of Ceftolozane and Tazobactum in bulk and pharmaceutical dosage form by RP-HPLC. Chromatograms were run isocratic way through the synchronis C_{18} column (250 mm x 4.6 mm, 5µm) using the mobile phase consists of methanol and 1% sodium perchlorate in the ratio of (80:20) and pumped through the column with pressure 10.5±5MPa at a flow rate of 1ml/min. The temperature was maintained at 30°C. The optimised wavelength for Ceftolozane and Tazobactum was 254 nm. Retention times of Ceftolozane and Tazobactum were found to be 4.65 min and 8.86 min respectively. % RSD of the Ceftolozane and Tazobactum were found to be 0.523 and 0.599 respectively. The % Recovery of Ceftolozane and Tazobactum at each level was found to be not less than 98.11 %, 98.03 and not more than 99.84%, 100.10% respectively. The percentage purity of Ceftolozane and Tazobactum in combined dosage form were found to be 98.13% and 98.67% respectively. The coefficient of variance for both the drug was more than 0.999. The method was validated in terms of linearity, precision, accuracy, limits of detection, limits of quantification, and robustness according to International Conference on Harmonization (ICH) guidelines. The proposed method can be used for routine quality control analysis of Ceftolozane and Tazobactum in bulk and combined dosage forms.

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INTRODUCTION

Zerbaxa is a combination of Ceftolozane/ tazobactam (Figure 1 and 2), which is novel antibacterial cephalosporin/β-lactamase inhibitor approved by the Food and Drug Administration (FDA) in the year 2014 (Xiao *et al*., 2015). Zerbaxa 1.5g (ceftolozane 1g and tazobactam 0.5g) is used for the treatment of complicated urinary tract infections as well as pyelonephritis in an adult caused by the Gram-negative microorganisms such as *Pseudomonas aeruginosa, Escherichia coli*, *Proteus mirabilis* and *Klebsiella pneumonia*. Zerbaxa is used with combination of metronidazole for the treatment of complicated intra-abdominal infections in the adult patients caused by the Gram-positive and Gram-negative microorganisms such as *Enterobacter cloacae*, *Klebsiella oxytoca, Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, Bacteroides *fragilis*, *Streptococcus anginosus*, *Streptococcus*

salivarius and *Streptococcus constellatus* (Sorbera *et al*., 2014; Giancola *et al*., 2016).

Various analytical methods were carried out for the estimation of Ceftolozane and Tazobactum as a single or combined with other drugs in pharmaceutical dosages such as estimation of Ceftolozane by UV and HPLC (Yashwanth and Shetty, 2011; Lalitha *et al*., 2008; Kumar and Dharuman, 2010; Dhandhapani and Rasheed, 2010); estimation of Tazobactum by UV and HPLC (Nanda *et al*., 2012; Kumar and Rao, 2010; Bhavana *et al*., 2013; Rao and Krishna, 2011; Amareswari *et al*., 2013; Sunitha and Sindhura, 2013; Marrapu and Kartheek, 2013; Gandhimathi *et al*., 2010); estimation of Ceftolozane and Tazobactum by HPLC(Swetha et al., 2017). 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.

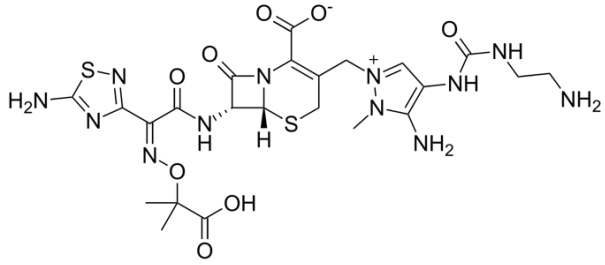


Figure 1: Structure of Ceftolozane

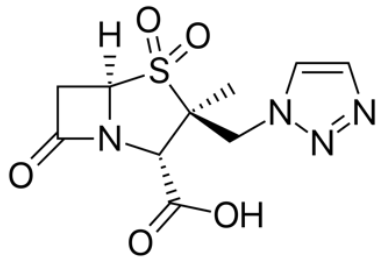


Figure 2: Structure of Tazobactam

MATERIALS AND METHODS

Instrumentation

Chromatographic separation was performed on a PEAK chromatographic system equipped with LC-P7000 isocratic pump; Rheodyne injector with 20 µl fixed volume loop, variable wavelength programmable UV detector UV7000 and the output signal was monitored and integrated by PEAK Chromatographic Software version 1.06. Teccomp UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis, and the data was recorded by Hitachi software. Sonicator (1.5L) was used to sonicating the mobile phase and samples. Standard and sample drugs were weighed by using Denver electronic analytical balance (SI-234), and pH of the mobile phase was adjusted by using Systronics digital pH meter.

Chromatographic conditions: Column C_{18} (250 mm × 4.6 mm); particle size packing 5 µm; detection wavelength of 245 nm; flow rate 1.00 ml/min; temperature ambient; sample size 20 µl; mobile phase methanol and 1% sodium perchlorate (80:20).

MATERIALS

Ceftolozane (CEF) and Tazobactum (TAZ) were obtained as a gift sample from Aurobindo Pharma, Hyderabad, India. Dry injectable powder of Cefoperazone and Tazobactam (Zerbaxa-Merck& Co., Inc) was purchased from a local pharmacy store. Sodium perchlorate and methanol were procured from Sigma Aldrich, Bangalore, India. Water and 0.45 µm filter (Millipore, Bangalore) were also used. All the reagents and chemicals used in the study were analytical grade.

Preparation of standard stock solution

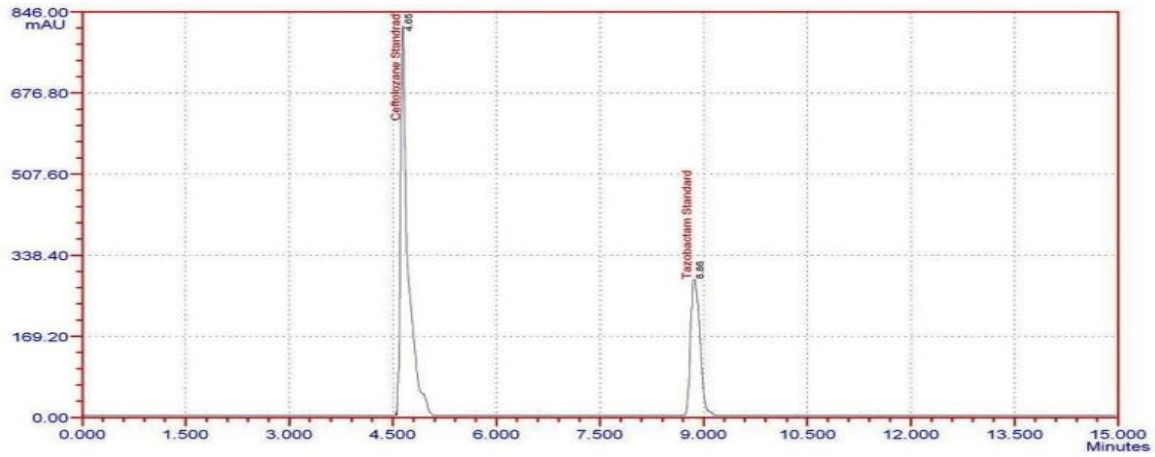
About 10 mg of Ceftolozane and 10 mg of Tazobactam sodium were weighed and separately taken into 100 ml volumetric flasks. To each flask 20 mL of methanol was added, sonicated for 10 min and then made up to the required volume with the methanol, to get a concentration of 100 µg/ml solutions. From this solution, 4 mL and 2 mL were taken into a 10 mL flask and made up to final volume with methanol to get a concentration of 40 µg/ml and 20 µg/ml respectively. The solution was filtered through the 0.45µ filter under vacuum filtration.

Preparation of the sample solution

11.25 mg of dry powder injection equivalent to about Ceftolozane was accurately weighed and taken into 100 ml volumetric flasks. To each flask 20 mL of methanol was added, sonicated for 10 min and then made up to required volume with the methanol, to get a concentration of 100 µg/ml of Ceftolozane and 50 µg/ml of Tazobactam. From this solution, 4 mL was taken into a 10 mL flask and made up to final volume with methanol to get a concentration of 40 µg/ml of Ceftolozane and 20 µg/ml of Tazobactam. The solution was filtered through the 0.45µ filter under vacuum filtration.

METHOD VALIDATION

Analytical method validation such as linearity, accuracy, limit of detection, limit of quantification, precision, robustness and system suitability studies were carried out as per the ICH guidelines (ICH, 2005).



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	Ceftolozane Standrad	4.647	82356	652110.4	69.185	1.10	6864
2	Tazobactam Standard	8.863	28998	290451.0	30.815	1.13	15606
	Sum:		111354	942561.4	100.0000		

Figure 3: Chromatogram of standard Ceftolozane and Tazobactam

RESULT AND DISCUSSION

Optimised Chromatographic Conditions

Ceftolozane is broad-spectrum antibiotic and Tazobactam is a β-lactamase inhibitor, both are used for the treatment of bacterial infections. The estimation was resolved by using ODS C_{18}, 5 µm, 250 x 4.6 mm, with mobile phases containing methanol and 1% sodium perchlorate (80:20) were quite satisfactory when used in the isocratic program. The flow rate was 1.0 ml/min and wavelength at 254 nm at which better detector response for drugs was obtained. The retention time for Ceftolozane and Tazobactam were found to be 4.65 min and 8.86 min respectively as shown in Table 1 and Figure 3.

Table 1: Optimized Chromatographic Conditions

S.NO	Parameter	Results
1	MP	Methanol: 1%sodium per-chlorate80:20 (v/v)
2	Wavelength	254nm
3	Stationary Phase	Synchronies C-18 (250mm x 4.6mm, 5µm) column
4	pH of MP	4.2 with 1% Perchloric acid
5	Flow Rate	1.0ml/min
6	Pump Mode	Isocratic
7	Pump Pressure	10.5±5MPa

METHOD VALIDATION

The developed method was validated for various parameters as per ICH guidelines like accuracy, Precision, linearity, specificity, ruggedness, robustness and solution stability.

Linearity

The linearity of both drugs was separately evaluated by analysing the standard solutions of Ceftolozane and Tazobactam in the range of 10–60 µg/ml and 5-30 µg/ml respectively and a calibration curve was plotted and constructed against concentration Vs Peak area. The result is shown in Table 1 and Figure 4, 5.

Table 2: Linearity of Ceftolozane and Tazobactam

Ceftolozane		Tazobactam	
Conc-entration	Peak area	Conc-entration	Peak area
10	174937	5	81259
20	331304	10	150080
30	507828	15	217872
40	652110	20	290451
50	842971	25	366338
60	1005312	30	443765

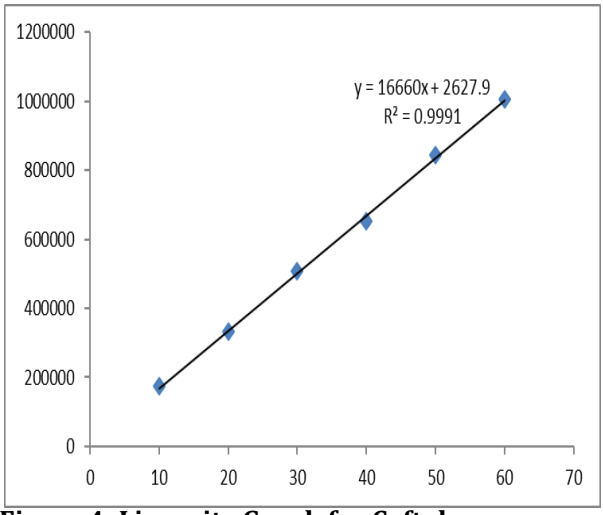


Figure 4: Linearity Graph for Ceftolozane

Table 5: Recovery study of Ceftolozane

S. No	Target	Spiked	Total	Amount found	% Recovery
1	20	10	30	29.83022	99.43406
2	20	10	30	29.44038	98.1346
3	20	10	30	29.83022	99.43406
4	20	20	40	39.78936	99.47339
5	20	20	40	39.77967	99.44916
6	20	20	40	39.93725	99.84312
7	20	30	50	49.84638	99.69275
8	20	30	50	49.05863	98.11725
9	20	30	50	49.60764	99.21528

Table 6: Recovery study of Tazobactam

S. No	Target	Spiked	Total	Amount found	% Recovery
1	10	5	15	14.70533	98.03554
2	10	5	15	14.86051	99.0701
3	10	5	15	14.79153	98.61019
4	10	10	20	20.02059	100.1029
5	10	10	20	19.88466	99.42331
6	10	10	20	19.88859	99.44294
7	10	15	25	24.87846	99.51384
8	10	15	25	24.65817	98.63268
9	10	15	25	24.8631	99.45242

Table 8: Robustness study of Ceftolozane and Tazobactam

Condition	Ceftolozane		Tazobactam	
	Area	% Change	Area	% Change
Standard	652110	.....	290451	....
MP 1	648746	0.514	288597	0.638
MP 2	648028	0.624	289894	0.192
pH 1	649831	0.348	289561	0.306
pH 2	649979	0.325	286204	1.462

Table 10: Assay of Ceftolozane and Tazobactam in dry power injection

S.no	Brand name	Available form	Dosage	Amount prepared	Amount found	% assay
1	Zerbaxa	Vial	Ceftolozane1.0g	40µg/ml	39.252µg/ml	98.129
2			Tazobactam 0.5g	20µg/ml	19.737µg/ml	98.688

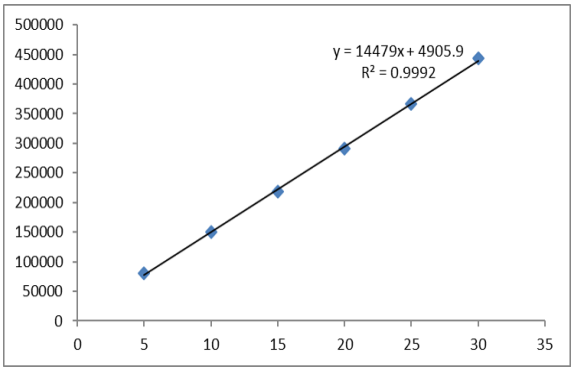


Figure 5: Linearity Graph for Tazobactam

Precision

The precision of the proposed method was carried out by making the repeated injections of a mixture of Ceftolozane and Tazobactam. The % RSD after six determinations was determined at 40 µg/ml for Ceftolozane and 20 µg/ml for Tazobactam. Intra-day and Inter-day precision of the reported method was estimated in term of % RSD. The estimation as repeated three times in a day for intraday precisian and three different days for interday precision by taking a concentration of 40 µg/ml of Ceftolozane and 20 µg/ml of Tazobactam**.** % RSD of intraday precision of Ceftolozane and Tazobactum were found to be 0.523, and 0.599 respectively are shown in Table 3 and % RSD of interday precision of Ceftolozane and Tazobactum were found to be 0.301, and 0.969 respectively are shown in Table 4.

Accuracy: Accuracy was calculated by the recovery studies at three levels by standard addition method, i.e. spiking 50%, 100%, 150% of Ceftolozane and Tazobactam. The % Recovery of Ceftolozane and Tazobactum at each level was found to be not less than 98.11 %, 98.03 and not more than 99.84%, 100.10% respectively as shown in Table 5 and 6.

Table 3: Intraday precision of Ceftolozane and Tazobactam

S No	Ceftolozane at 40µg/ml	Tazobactam at 20µg/ml
1	652110	290451
2	658748	294566
3	658758	291558
4	655984	294114
5	650438	290857
6	654287	291304
RSD	0.523	0.599

Table 4: Interday precision of Ceftolozane and Tazobactam

S No	Ceftolozane at 40µg/ml	Tazobactam at 20µg/ml
1	654598	295536
2	652219	289296
3	650388	290039
4	652931	295203
5	650099	294264
6	654602	295345
RSD	0.301	0.969

LOD and LOQ

LOD and LOQ of Ceftolozane and Tazobactam were determined by calibration curve method. Solutions of Ceftolozane and Tazobactam were prepared in the range of 5–15 µg/ml and 1-7 µg/ml and injected in triplicate. The LOQ and LOD of Ceftolozane and Tazobactam were found to be 0.25µg/ml, 0.075µg/ml and 0.13µg/ml, 0.005µg/ml respectively as shown in Table 7.

Table 7: LOQ and LOD value of Ceftolozane and Tazobactam

Drug	LOQ	LOD
Ceftolozane	0.25µg/ml	0.075µg/ml
Tazobactam	0.13µg/ml	0.005µg/ml

Robustness: Influence of small changes in chromatographic conditions such as a change in mobile phase composition (±2 ml) and pH (± 0.2) was studied to determine the robustness of the method for the development of RP-HPLC method for the simultaneous estimation of Ceftolozane and Tazobactam was determined as shown in Table 8.

System Suitability: System Suitability, the stock solution containing 40 µg/ml of Ceftolozane and 20 µg/ml of Tazobactam was injected and repeated five times, and the chromatograms were recorded. The resolution, number of theoretical plates, and peak asymmetry were calculated to determine whether the result complies with the recommended limit as shown in Table 9.

Assay: The assay of the developed method was applied for the analysis of Zerbaxa dry powder injection, and the results of the assay of Ceftolozane and Tazobactam are presented in Table 10.

Table 9: System Suitability Conditions

S.No	Parameter	Results
1	API Concentration	Ceftolozane – 40µg/ml Tazobactam - 20µg/ml
2	RT	Ceftolozane – 4.65min Tazobactam -8.86min
3	Resolution	Ceftolozane – 15.51 Tazobactam – 16.60
4	Area	Ceftolozane – 652110 Tazobactam - 290451
5	Theoretical Plates	Ceftolozane – 6864 Tazobactam - 15606
6	Tailing Factor	Ceftolozane – 1.10 Tazobactam - 1.13

CONCLUSION

The proposed RP-HPLC (Reverse Phase High-performance Liquid Chromatography) method has been evaluated for the accuracy, precision and linearity. The method was found to be precise, accurate and linear over the linear concentration range. The analytical method validation of Ceftolozane and Tazobactam by RP-HPLC was found to be satisfactory and could be used for the routine pharmaceutical analysis of Ceftolozane and Tazobactam. The method was validated as per ICH guidelines like system suitability, accuracy, precision, linearity, specificity, ruggedness, robustness and solution stability, Therefore, this HPLC method can be used as a routine analysis of these drugs in bulk, pharmaceutical formulations and also for stability studies.

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