https://doi.org/10.48047/AFJBS.6.7.2024.3245-3262

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF CEFTOLOZANE AND TAZOBACTAM IN BULK AND PHARMACEUTICAL DOSAGE FORM

Amol M. Shirode[1], Rutuja K. Suryawanshi[2]*, Vinod A. Bairagi[3], Ganesh B. Sonawane[4], Pravin B. Jadhav[1]

[1]Department of Pharmaceutical Chemistry, K.B.H.S.S. Trust's Institute of Pharmacy Malegaon, Nashik-423105, Maharashtra, India

[2]Department of Pharmaceutical Quality Assurance, K.B.H.S.S Trust's Institute of Pharmacy, Malegaon, Nashik-423301, Maharashtra, India

[3]Department of Pharmacology, K.B.H.S.S. Trust's Institute of Pharmacy Malegaon, Nashik-423105, Maharashtra, India

[4]Department of Pharmaceutical Chemistry, Divine College of Pharmacy, Satana, Nashik-423301, Maharashtra, India

Corresponding Author Email: rutujasuryawanshi225@gmail.com

Volume 6 issue 7 2024

Received:01June2024

Accepted:30June2024

doi:10.48047/AFJBS.6.7.2024.3245-3262

Abstract

A precise and robust method was developed method for the estimation of Ceftolozane and Tazobactam in pharmaceutical dose form and bulk. Agilent 1260 Infinity II model HPLC with DAD detector and Phenomenex Kinetex XB-C8 with dimensions of 150 x 4.6 mm, 5 µm was utilized in the method. The Mobile phase combination used was 0.1% Trifluoroacetic acid and Acetonitrile (70:30). The retention time of Ceftolozane (TOL) and Tazobactam (TAZ) peaks was at 3.05 and 3.96 minutes, respectively. In compliance with ICH Q2 (R1) requirements, the devised approach was validated. The instrument precision for TOL & TAZ had a %RSD of 0.66% and 0.68%, respectively. The Intra & Inter day precision for TOL & TAZ had a %RSD of 0.74% and 0.24%, respectively The linear and accurate method was applied to concentration ranges of 80-120 µg/ml and 40-60 µg/ml for both TOL and TAZ, respectively. The regression coefficient for both TOL and TAZ was 0.999 and % RSD for accuracy for TOL at 80%, 100% and 120% was found to 0.82%, 0.93% and 0.12% respectively; and for TAZ at 80%, 100% and 120% was found to be 0.33%, 0.89% and 0.55% % respectively. The LOD & LOQ for TOL are 1.65 µg/ml and 4.99 µg/ml respectively and the LOD & LOQ for TAZ are 1.41 µg/ml and 4.26 µg/ml respectively.

Keywords: Ceftolozane, Tazobactam, antibiotic, RP-HPLC, Robustness, %RSD, Precision, LOD, LOQ, Accuracy, etc.

1. INTRODUCTION

The most effective antipseudomonal agent available at the moment is ceftolozane-tazobactam, which is effective against multidrug-resistant and extensively drug-resistant strains of the bacteria. Tazobactam exhibits increased action against a wide variety of beta-

lactamases that have an extended spectrum of activity against Enterobacterales [1]. Specifically, ceftolozane-tazobactam has been granted formal approval for the treatment of difficult urinary tract infections, complex intra-abdominal infections, hospital-acquired bacterial pneumonia, and ventilator-associated bacterial pneumonia [2]. Ceftolozane-tazobactam, also known as TOL-TAZ, is a combination of a traditional β-lactamase inhibitor (tazobactam) with a newly developed antipseudomonal cephalosporin (ceftolozane) that possesses increased antipseudomonal activity [3]. It is possible for TOL-TAZ to inhibit extended-spectrum beta-lactamases (ESBL) as well as class A serine-betalactamases. Additionally, TOL-TAZ is effective against non-ESBL class D oxacillinases; however, it does not exhibit any activity against carbapenemases [4].

Ceftolozane/tazobactam is recommended for the treatment of infections in adults that are caused by bacteria that have been identified as susceptible to the medication including:

- Infections that are complicated within the abdominal cavity;
- Acute Pyelonephritis
- Complicated Infections of the urinary tract
- Hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia (also known as HABP and VABP) [5, 6]

The IUPAC name of Ceftolozane is (6R,7R)-3-[[3-amino-4-(2-aminoethylcarbamoylamino)-2-methylpyrazol-1-ium-1-yl]methyl]-7-[[(2Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(2-carboxypropan-2-yloxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [7]. The compound known as ceftolozane is composed of a 7-aminothiadiazole, which enables it to exhibit enhanced action against gram-negative organisms. Additionally, it possesses an alkoximino group, which ensures its stability against a wide range of β-lactamases. Through the utilization of a pyrazole ring, which is a bulky side chain, the β-lactam ring is prevented from being hydrolyzed by the inclusion of a pyrazole ring at the 3-position [8].

The IUPAC name of Tazobactam is (2S,3S,5R)-3-methyl-4,4,7-trioxo-3-(triazol-1-ylmethyl)-4λ6-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid [9]. Tazobactam, which is classified as a penicillinate sulfone β-lactamase inhibitor, is a compound that inhibits the hydrolysis of the amide link of β-lactam molecules by enzymes that are members of the β-lactamase family [10].

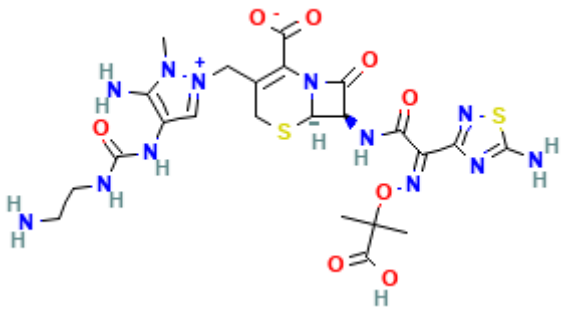


Figure 1: Structure of Ceftolozane

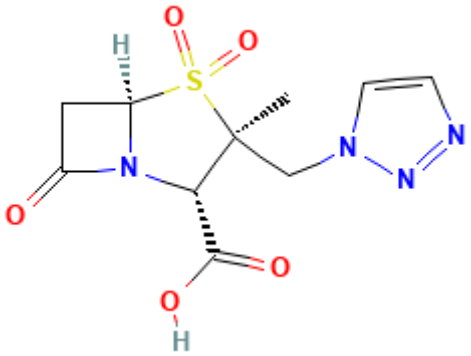


Figure 2: Structure of Tazobactam

According to the study of the relevant literature [11-23], there were only a few liquid chromatography analyses that were performed for simultaneous determination of TOL and TAZ in combination pharmaceutical dose form. This study was planned with the purpose of developing and validating a method that had been devised for the treatment of ceftolozane and Tazobactam.

Table 1: Quality Target Profile for HPLC Method development

Parameter	Limits
Theoretical Plates	Not less than 2000
Asymmetry	Not More than 2.0 (Fairly at 1.0)
Tailing Factor	Not More than 2.0 (Fairly at 1.0)
Run time	Not More than 20 minutes
Resolution	Not Less than 2.0

2. MATERIAL AND METHOD

2.1. Chemicals and Reagents

A complimentary sample of ceftolozane and Tazobactam was made available by Aadhaar Life Sciences Pvt. Ltd. Acetonitrile was acquired from Qualigens in India and was of HPLC grade. AR grade Trifluoroacetic acid was purchased from Merck in India. The company was located in India. The water was supplied via the internal Milli-Q system. NABL scales that had been calibrated were used for all of the weighing. The analytical balance was utilized in the production of the samples, which were made in Type A glassware.

2.2. Instrumentation

Agilent 1260 Infinity II, equipped with a quaternary pump and DAD detector, served as the development and validation instrument. Openlab Ezchrom software from Agilent was used. For wet chemistry, we used the Aczet analytical balance and the Labman ultrasonicator.

2.3. HPLC Method Development

2.3.1. The table 2 and 3 describes trials done during the development phase with the results and observations.

Table 2: Method Development for Ceftolozane & Tazobactam HPLC

Trial No.	Mobile Phase	Ratio	Diluent	Column	Flow rate	Wavelength
1	0.1% TFA acid:ACN	50-50	50 0.1% TFA acid: 50ACN	Phenomenex Kinetex XB-C8 (150 x 4.6mm, 5µm)	1.0 ml/min	250 nm
2	0.1% TFA acid:ACN	60-40	50 0.1% TFA acid: 50ACN	Phenomenex Kinetex XB-C8 (150 x 4.6mm, 5µm)	1.0 ml/min	212 nm
3	0.1% TFA acid:ACN	70-30	50 0.1% TFA acid: 50ACN	Phenomenex Kinetex XB-C8 (150 x 4.6mm, 5µm)	1.0 ml/min	212 nm
4	0.1% TFA acid:ACN	70-30	50 0.1% TFA acid: 50ACN	Phenomenex Kinetex XB-C8 (150 x 4.6mm, 5µm)	0.5 ml/min	212 nm

Table 3: Method development results of Ceftolozane and Tazobactam

Trial No.	Mobile Phase	Ratio	Diluent	Column	Flow rate	Wavelength
1	0.1% TFA acid:ACN	50-50	50 0.1% TFA acid: 50ACN	Phenomenex Kinetex XB-C8 (150 x 4.6mm, 5µm)	1.0 ml/min	250 nm
2	0.1% TFA acid:ACN	60-40	50 0.1% TFA acid: 50ACN	Phenomenex Kinetex XB-C8 (150 x 4.6mm, 5µm)	1.0 ml/min	212 nm

3	0.1% TFA acid:ACN	70-30	50 0.1% TFA acid: 50ACN	Phenomenex Kinetex XB-C8 (150 x 4.6mm, 5µm)	1.0 ml/min	212 nm
4	0.1% TFA acid:ACN	70-30	50 0.1% TFA acid: 50ACN	Phenomenex Kinetex XB-C8 (150 x 4.6mm, 5µm)	0.5 ml/min	212 nm

The highest absorption of both peaks was determined to be at 212 nm in all of the above trials. The diluent used for all trails was kept constant at 50-50 0.1% Trifluroacetic acid-Acetonitrile. The Phenomenex Kinetex XB-C8 (150 x 4.6 mm, 5 micron) column was utilized in all of the studies. The conditions for trial 4 were established based on the predefined quality target profile for development work, and an individual Standard was run to validate the retention times. Figure 3 displayed the chromatograms of the method development.

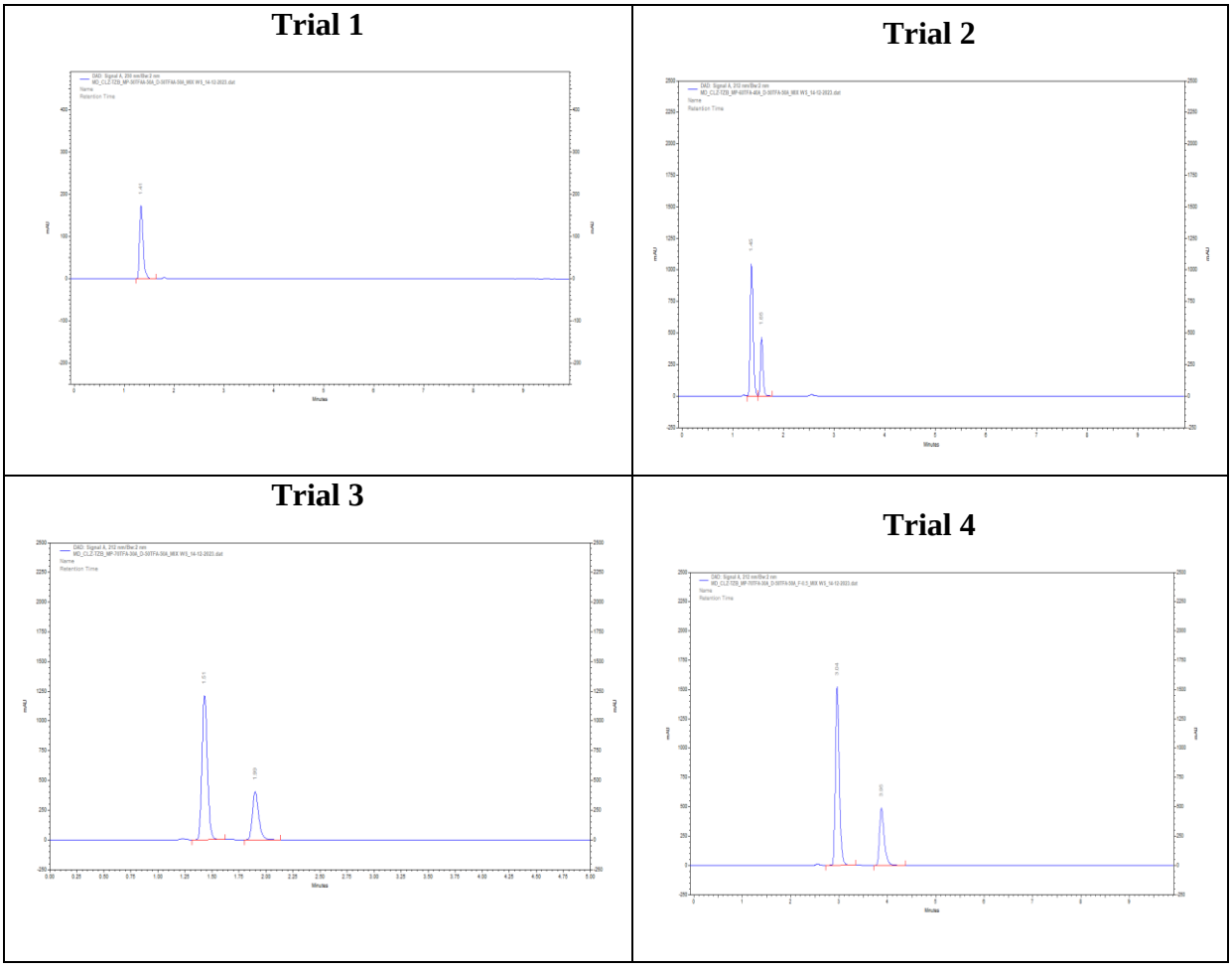


Figure 3: Method Development Trials

2.3.2. Final Chromatographic Conditions:

Table 4: Final Chromatographic Condition

Parameter	Condition
HPLC Instrument	Agilent 1260 Infinity II
Column	Phenomenex Kinetex XB-C8 (150 mm x 4.6 mm, 5µm)
Wavelength	212 nm
Mobile Phase	Mobile Phase A –0.1% Trifluroacetic acid: 70% Mobile Phase B – Acetonitrile : 30%
Diluent	0.1% Trifluroacetic acid : Acetonitrile (50:50) v/v
Run time	10 minutes
Injection Volume	10 micro liters
Flow Rate	0.5 ml/min
Column oven Temperature	30°C (± 2°C allowed by Robustness)

2.3.3. Preparation of Mobile Phase

Preparation of 0.1% Trifluoroacetic acid

Using a graduated cylinder, take 800 ml of water. After measuring the water and adding 1 ml of trifluroacetic acid, thoroughly mix the mixture and use water to adjust the content to 1000 ml.

Mobile Phase: 70%- 0.1% Trifluoroacetic acid: 30% Acetonitrile

Mix separately measured 700 ml of 0.1% Trifluoroaceti**c** acid and 300 ml of Acetonitrile into a suitable container. Filter the mobile phase through 0.45 µm nylon membrane filter. Briefly sonicate to degas.

2.3.4. Preparation of Diluent

500 ml of Trifluoroacetic acid and 500 ml of acetonitrile, measured separately, should be thoroughly mixed in a suitable container. A 0.45 µm nylon membrane filter is to be used to filter the mixture. To degas, briefly sonicate.

2.3.5. Preparation of Standard Solution

A. Working Standard:

1. **Ceftolozane Standard Stock Solution-I (CSSS-I):**
    i. Initially Prepare a Standard Stock Solution (SSS-I) of by adding 10 mg of Ceftolozane in 10 ml volumetric flask & add 5 ml diluent, mix

for 2 minutes and make the volume to 10 ml with diluent (Conc. of Ceftolozane= 1000 µg/ml).

2. **Tazobactam Standard Stock Solution-I TSSS-I):**
    i. Then prepare a Standard Stock Solution (SSS-II) of Tazobactam by adding 10 mg in 10 ml volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent (Conc. of Tazobactam = 1000 µg/ml).
3. Then add 1.0 ml of CSSS-I & 0.5 ml TSSS-I in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent (Conc. of Ceftolozane= 100 µg/ml & Tazobactam = 50 µg/ml).

B. Preparation of Sample for Assay

1. EXTACEFE-TAZO Injection were used as marketed product

2. Weigli powder equivalent to 10 mg of Ceftolozane and 3 mg of Tazobactam and transfer to. 10 ml volumetric flask & add 5-7 ml diluent, mis for 5 minutes and make the volume to 10 ml with diluent. (Conc. of Ceflolozane = 1000mug /ml and Tazobactam = 50mug/ml.

3. Then add 1.0 ml of above stock solution in 10 ml vohimetric flask and add 5 ml diluent and vortex and make up the volume with diluent (Cone of Ceftolozane 100 pa/ml & Tazobactam = 50mug / mD.

2.4. Method validation

2.4.1. Specificity

Peaks were found in the Retention Time for individual injections of Ceftolozane and Tazobactam, which were produced at 100 µg/ml and 50 µg/ml, respectively. In order to make sure that the primary analyte peaks are not being interfered with, blank was injected.

2.4.2. System Suitability

The system's suitability and performance were assessed using a variety of tests. Theoretical Plate count, tailing factor, and resolution are all found to be within allowed ranges for the ICH guideline system

2.4.3. Accuracy

A technique's accuracy can be assessed by looking at how closely the results of its tests match the real value. Three different concentration levers have been evaluated in the recovery

studies. Three replicate injections were made at each level, and the amount of drug present, the recovery percentage, and the associated standard deviation were calculated.

2.4.4. Repeatability

The degree of concordance between individual test findings determines analytical precision. A homogeneous sample was evaluated in multiple samples. Six injections were made from the same sample, which was prepared as per the instructions, and the system's appropriateness was verified. The instrument's precision (i.e., its ability to perform back-to-back replicate injections of the same concentration) was assessed.

2.4.5. Linearity

The capacity of an analytical method to produce results that are proportionate to analyte concentrations within a certain range is referred to as the methodological linearity of the method. When determining linearity, there were five different sets of standard solutions that were utilized. The regression equation was established by plotting the peak area against the concentration of the standard solution on the calibration curve. This allowed for the development of the equation. For the purpose of determining the slope, intercept, and correlation coefficient, the least-squares method was utilized.

2.4.6. LOD and LOQ

The methods' ability to detect and quantify the smallest amount of analyte is indicated by the LOD and LOQ, respectively. The following formulas were used to calculate the LOD and LOQ using the standard deviation and regression line slope.

2.4.7. Robustness

The Robustness was performed changing the column temperature by ± 2˚C and Wavelength by ± 2 nm.

Table 5: Robustness Trials

Condition	**Increased**	**Normal**	**Decreased**
Column Oven Temperature	32˚C	30˚C	28˚C
Wavelength	214 nm	212 nm	210 nm

2.4.8. Inter-day & Intraday Precision:

To determine the stability of the solution for intraday precision, the prepared working standard was analyzed in the morning and the evening, and the percentage RSD was calculated. On the second day, the identical solution was injected, and the percentage RSD was determined by comparing the morning intraday precision values.

3. RESULTS AND DISCUSSION

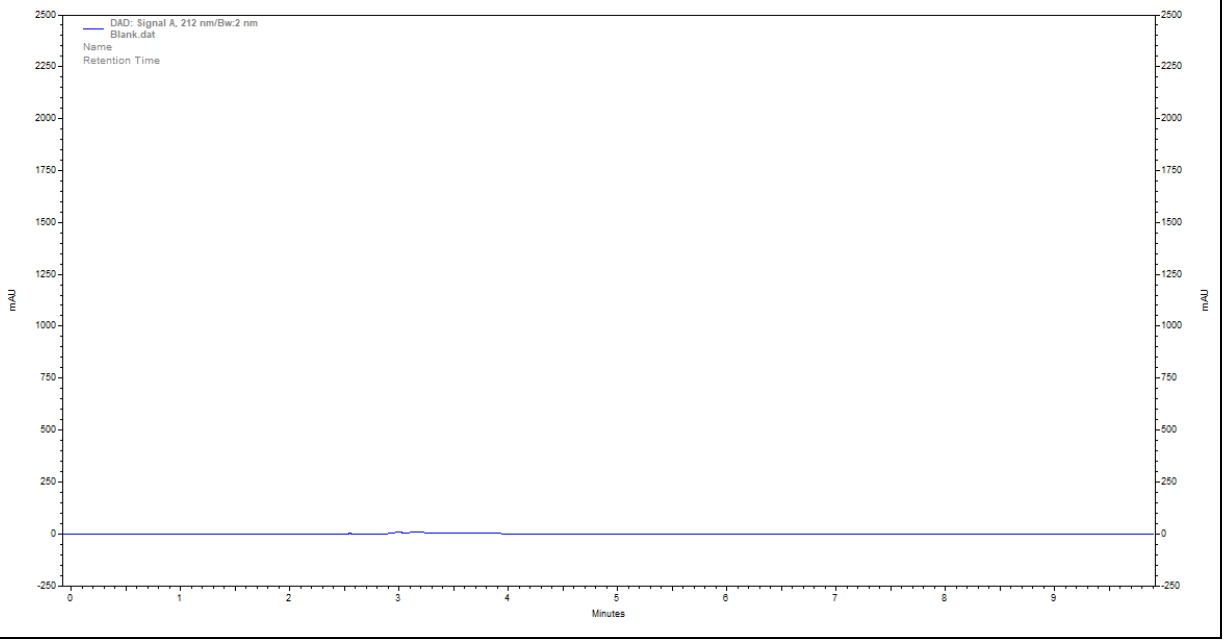
3.1. Specificity

Specificity was performed to check if there was any interaction between the peaks from blank or the APIs.

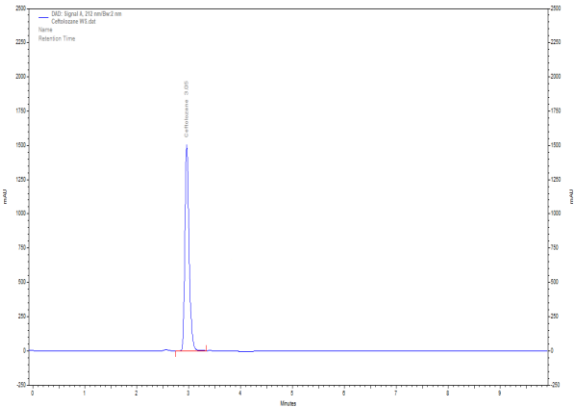
Table 6: Specificity results of Ceftolozane and Tazobactam

Sample	Ceftolozane			Tazobactam		
	RT	Area	% Assay	RT	Area	% Assay
Ceftolozane	3.05	17735697	-	-	-	-
Tazobactam	-	-	-	3.96	6877445	-
MIX WS	3.05	17748689	-	3.96	6878050	-
Drug Product	3.05	17665212	99.53	3.96	6865541	99.82

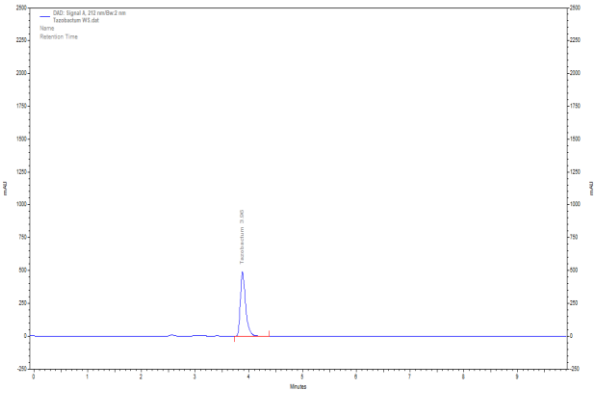
a. Diluent



b. Ceftolozane WS



c. Tazobactam WS



d. Mix WS

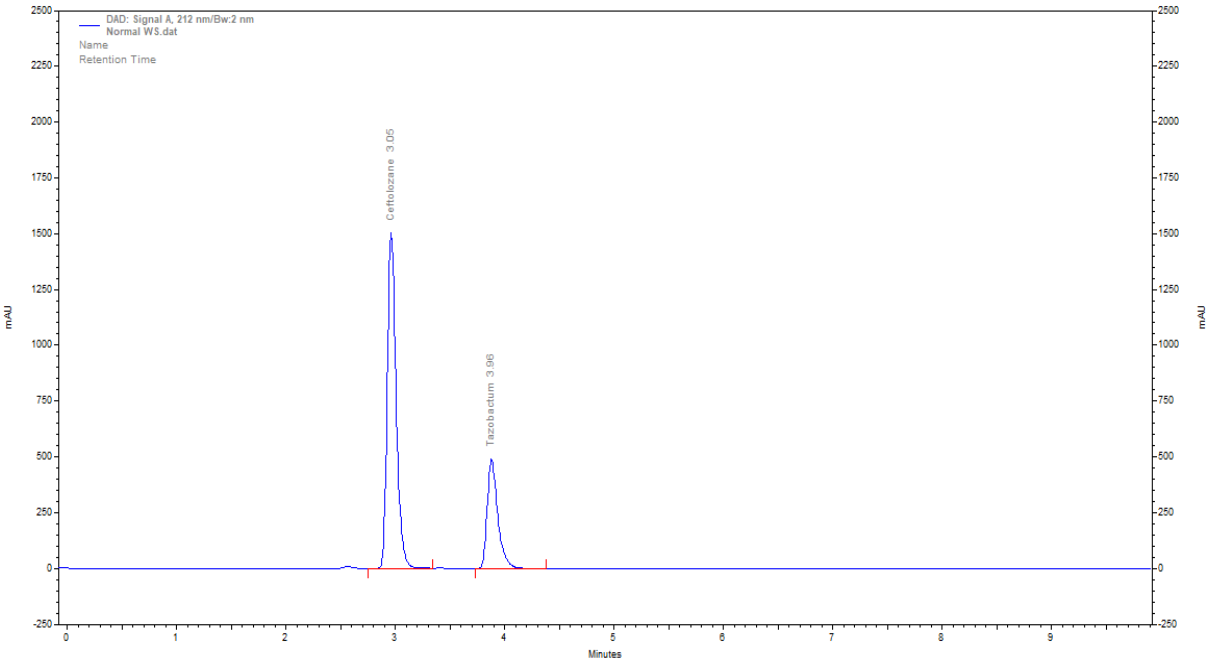


Figure 4: Chromatogram ID a] Diluent, b] Ceftolozane c] Tazobactam, d] Mixture Working Standard of TOL & TAZ

3.2. Instrument Precision and System suitability

The HPLC instrument's suitability for carrying out the validation was examined through testing. The equipment was determined to be suitable for carrying out the validations based on the restrictions listed in Table 1. The relative standard deviation for the instrument precisions of TOL and TAZ are 0.66% and 0.68%, respectively, according to the data presented below. The instrument precisions of both medications were carried out following system appropriateness. The method's high degree of precision in preparing several samples

at the same concentration is demonstrated by this %RSD. Table 7-9 displays the information.

Table 7: System suitability for Ceftolozane

Ceftolozane				
Reps	**RT**	**Asymmetry**	**Theoretical Plates**	**Resolution**
Rep 1	3.05	1.26	7031	0.00
Rep 2	3.05	1.28	7114	0.00
Rep 3	3.05	1.22	7158	0.00
Rep 4	3.05	1.21	7248	0.00
Rep 5	3.05	1.18	6922	0.00
Rep 6	3.05	1.28	7011	0.00
Avg	3.05			
STDEV	0.00			
RSD	0.00			

Table 8: System suitability for Tazobactam

Tazobactum				
Reps	**RT**	**Asymmetry**	**Theoretical Plates**	**Resolution**
Rep 1	3.96	1.53	8533	5.77
Rep 2	3.96	1.47	8562	5.77
Rep 3	3.96	1.52	8518	5.77
Rep 4	3.96	1.49	8725	5.77
Rep 5	3.96	1.55	8633	5.77
Rep 6	3.96	1.54	8432	5.77
Avg	3.96			
STDEV	0.00			
RSD	0.00			

Table 9: Instrument precision of Ceftolozane and Tazobactam

Repeatability		
Sample ID	**Peak area**	
	Ceftolozane	**Tazobactam**
100% Rep 1	17748689	6878050
100% Rep 2	17422256	6756997
100% Rep 3	17568845	6812123
100% Rep 4	17655123	6785521
100% Rep 5	17712565	6865442
100% Rep 6	17636542	6819556
AVG	174624003	6819615
STDEV	116841.57	46161.264
%RSD	0.66	0.68

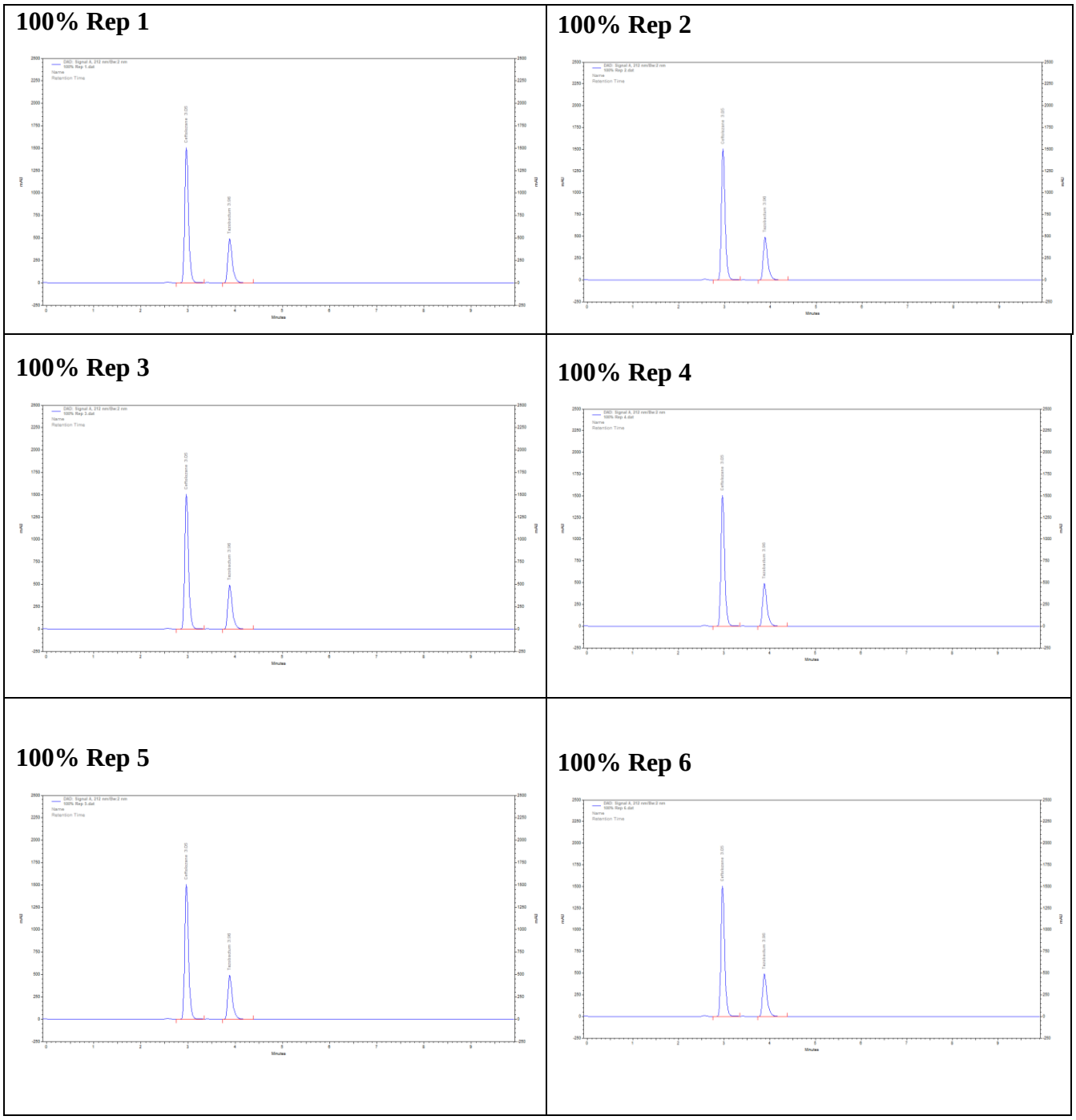


Figure 5: Instrument Precision Ceftolozane & Tazobactam

3.3. Linearity of Ceftolozane & Tazobactam

At different levels, linearity was tested. The graph plotted between peak area and concentration showed linearity with correlation coefficient as shown in table below. Table 10 and the graph in Figure 6 display the linearity results.

Table 10: Linearity data of TOL & TAZ

Ceftolozane			Tazobactam		
% Level	**Conc (ug/ml)**	**Area**	**% Level**	**Conc (ug/ml)**	**Area**
80	80	14312337	80	40	5500083
90	90	16086991	90	45	6202597
100	100	17748689	100	50	6878050
110	110	19486837	110	55	7532142
120	120	21215066	120	60	8253117
R^2= 0.999			R^2= 0.999		

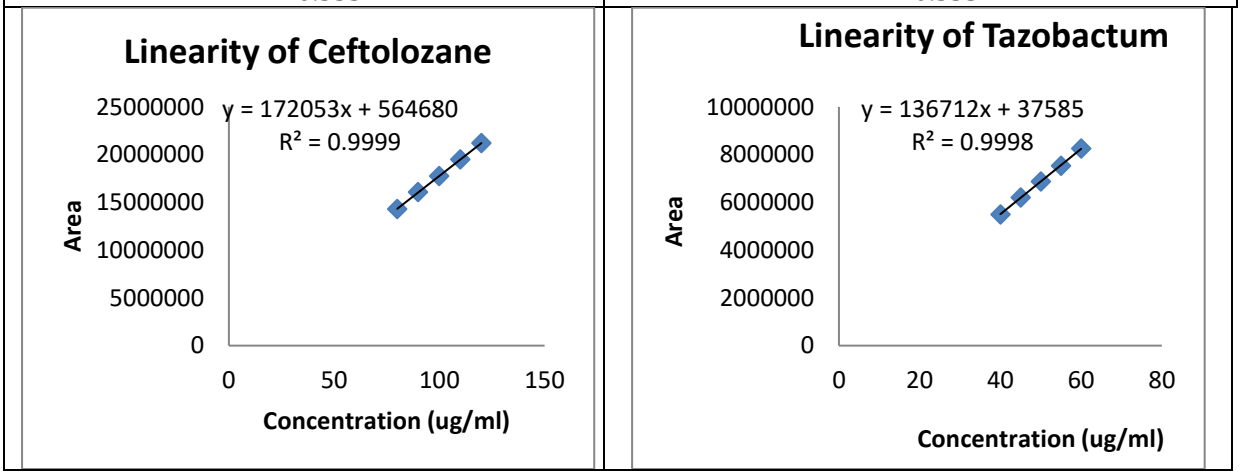


Figure 6: Linearity graph of Ceftolozane and Tazobactam

3.4. LOD and LOQ for Ceftolozane and Tazobactam

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined for CEF & TAZ. The results of analysis are shown in table 11.

Table 11: LOD and LOQ for TOL & TAZ

Name	LOD (µg/ml)	LOQ (µg/ml)
Ceftolozane	1.65	4.99
Tazobactam	1.41	4.26

Given that the LOD and LOQ were both very low, it can be concluded that the method used is highly effective at identifying low drug concentrations. Businesses can utilize the values of LOD and LOQ to determine whether manufactured vessels or equipment are free of stains from APIs during the cleaning validation process.

3.5. Accuracy

The technique proved to be accurate for the range of 80%, 100%, and 120% when the accuracy for TOL was tested in triplicate. In relation to 80%, 100%, and 120%, the %RSD were found to be 0.82%, 0.93%, and 0.12%. The precision established the method's ability to precisely analyze various drug concentrations in solution. Table 12 displays the accuracy data.

Table 12: Accuracy data for Ceftolozane

Sample ID	Reps	Spiked Conc (ug/ml)	Area	Amount Recovered (ug/ml)	% Recovery	AVG	STDEV	%RSD
80%	Rep 1	79.76	14312337	80.97	101.51	101.12	0.826464	0.82
	Rep 2		14123648	79.90	100.17			
	Rep 3		14336447	81.10	101.68			
100%	Rep 1	99.70	17748689	100.41	100.71	99.75	0.927704	0.93
	Rep 2		17422256	98.56	98.86			
	Rep 3		17568845	99.39	99.69			
120%	Rep 1	119.64	21215066	120.01	100.31	100.26	0.124021	0.12
	Rep 2		21223657	120.06	100.35			
	Rep 3		21174545	119.79	100.12			

Accuracy for TAZ was performed in triplicates and it was observed that the method was accurate for the range 80%, 100% and 120%. The relative standard deviation for 80%, 100% and 120% were 0.33%, 0.89% and 0.55% respectively. The accuracy determined the methods ability to analyses different concentration of drug in solution accurately. The accuracy data is shown in table 13.

Table 13: Accuracy data for Tazobactam

Sample ID	Reps	Spiked Conc (ug/ml)	Area	Amount Recovered (ug/ml)	% Recovery	AVG	STDEV	%RSD
80%	Rep 1	39.88	5500083	40.20	100.81	100.56	0.333278	0.33
	Rep 2		5493312	40.15	100.69			
	Rep 3		5465755	39.95	100.18			
100%	Rep 1	49.85	6878050	50.28	100.86	99.94	0.888712	0.89
	Rep 2		6756997	49.39	99.08			
	Rep 3		6812123	49.80	99.89			
120%	Rep 1	59.82	8253117	60.33	100.85	100.25	0.556267	0.55
	Rep 2		8195567	59.91	100.15			
	Rep 3		8163245	59.67	99.75			

3.5. Inter and Intraday Precision

Intra and inter day precision study was performed and reported the % RSD change in peak area of the APIs at different time points. The acceptance criteria is to have %RSD of peak area <2%. The Results are given in Table 14.

Table 14: Intra & Interday Precision of TOL & TAZ

Intra Day precision					
Day 1	**Sample ID**	**Ceftolozane**		**Tazobactam**	
		Area	**Assay**	**Area**	**Assay**
Morning	**WS**	17748689	-	6878050	-
	DP	17665212	99.53	6865541	99.82
Evening	**WS**	17655562	-	6756332	-
	DP	17436842	98.76	6732115	99.64
Inter Day precision					
Day	**Sample ID**	**Ceftolozane**		**Tazobactam**	
		Area	**Assay**	**Area**	**Assay**
Day 2	**WS**	17526547	-	6796322	-
	DP	17455865	99.60	6752154	99.35
%RSD		0.47		0.24	

3.6. Robustness

It is necessary to perform robustness testing in order to determine the degree to which the approach deviates from its essential parameters. Before being used, the apparatus is calibrated in every region of the world; however, in order to determine whether or not the procedure is reliable, modifications were made to the column temperature and the wavelength, as indicated in tables 15 and 16.

Table 15: Robustness study - Change in Column temperature

Column Oven Temp Change					
Condition	**Sample**	**Ceftolozane**		**Tazobactum**	
		Area	**Assay**	**Area**	**Assay**
28°C	**WS**	17598856	-	6774225	-
	DP	17495565	99.41	6756662	99.74
30℃	**WS**	17748689	-	6878050	-
	DP	17665212	99.53	6865541	99.82
32℃	**WS**	17563321	-	6875152	-
	DP	17375561	98.93	6854222	99.70

Table 16: Robustness study - Change in Wavelength

Wavelength (nm)					
Condition	**Sample**	**Ceftolozane**		**Tazobactum**	
		Area	**Assay**	**Area**	**Assay**
210	**WS**	17736695	-	6796336	-
	DP	17633548	99.42	6789545	99.90
212	**WS**	17748689	-	6878050	-
	DP	17665212	99.53	6865541	99.82
214	**WS**	17766589	-	6864885	-
	DP	17566325	98.87	6859575	99.92

As a result, it was found that the technique remained robust even when the wavelength and column temperature changed slightly. The area of the replicate injection and retention time did not alter significantly.

4. CONCLUSION

In this research article, a method that is both precise and accurate was developed based on a technique that was developed for the purpose of estimating TOL and TAZ in bulk pharmaceuticals and formulations using the RP-HPLC technology. Validation was performed on the created approach to ensure that it is accurate, precise, and resilient. Because of their ease of use, dependability, sensitivity, speed, and selectivity for detection at extremely low concentrations, the methods that were developed were deemed to be suitable. When it comes to the routine analysis of ceftolozane and Tazobactam in a variety of formulations, these methods are able to be utilized because the validation data reveals that they are accurate, precise, straightforward, and cost-effective.

REFERENCES

1. Yahav D, Giske CG, Grāmatniece A, Abodakpi H, Tam VH, Leibovici L. New β-Lactam–β-Lactamase Inhibitor Combinations. Clin Microbiol Rev 2020;34(1):e00115-20. doi: 10.1128/CMR.00115-20.
2. Horcajada JP, Montero M, Oliver A, et al.. Epidemiology and Treatment of Multidrug-Resistant and Extensively Drug-Resistant Pseudomonas aeruginosa Infections. Clin Microbiol Rev 2019; 32(4):e00031-19. doi: 10.1128/CMR.00031-19.

3. Long TE, Williams JT (October 2014). "Cephalosporins currently in early clinical trials for the treatment of bacterial infections". Expert Opinion on Investigational Drugs. **23** (10): 1375–87.
4. Cho JC, Fiorenza MA, Estrada SJ. Ceftolozane/Tazobactam: A Novel Cephalosporin/ β-Lactamase Inhibitor Combination. Pharmacother J Hum Pharmacol Drug Ther 2015; 35:701–715. Doi: 10.1002/phar.1609.
5. "Zerbaxa EPAR". European Medicines Agency (EMA). 17 September 2018. Retrieved 14 May 2020. Text was copied from this source which is © European Medicines Agency. Reproduction is authorized provided the source is acknowledged.
6. "Zerbaxa- ceftolozane and tazobactam injection, powder, lyophilized, for solution". DailyMed. 3 June 2019. Retrieved 14 May 2020.
7. National Center for Biotechnology Information (2024). PubChem Compound Summary for CID 53234134, Ceftolozane. Retrieved February 8, 2024 from https://pubchem.ncbi.nlm.nih.gov/compound/Ceftolozane.
8. Murano K, Yamanaka T, Toda A, Ohki H, Okuda S, Kawabata K, et al. (March 2008). "Structural requirements for the stability of novel cephalosporins to AmpC beta-lactamase based on 3D-structure". Bioorganic & Medicinal Chemistry. **16** (5): 2261–75.
9. National Center for Biotechnology Information (2024). PubChem Compound Summary for CID 123630, Tazobactam. Retrieved February 8, 2024 from https://pubchem.ncbi.nlm.nih.gov/compound/Tazobactam.
10. Drawz SM, Bonomo RA (January 2010). "Three decades of beta-lactamase inhibitors". Clinical Microbiology Reviews. **23** (1): 160–201.
11. Ezquer-Garin, Carlos, et al. "Validated HPLC-UV detection method for the simultaneous determination of ceftolozane and tazobactam in human plasma." Bioanalysis 10.7 (2018): 461-473.
12. Kratzer, Alexander, et al. "Determination of total and free ceftolozane and tazobactam in human plasma and interstitial fluid by HPLC-UV." Journal of Pharmaceutical and Biomedical Analysis 163 (2019): 34-38.
13. Krantikumar, Perli, et al. "A Study On Method Development And Validation Of Drugs Used In Hospital Acquired Bacterial Pneumonia." Journal of Engineering Sciences 13.12 (2022).
14. M. A. Campanero, A.M.Zamarreno, M. Simon, M. C. Dios, J.R. Azanza. Simple and rapid etermination of piperacillin and ceftazidime in human plasma samples by

HPLC. Chromatographia: An IntenationalJouranal for Rpaid Communication in Chromatography, Electrophoresis and Associated Techniques. 46(7-8): 374-380 (1997).

15. R. Denooz C. Charlier. Simultaneous determination of five B-lactam antibiotics (cefepim, ceftazidime, cefuroxim, meropenem and piperacillin) in human plasma by high-performance liquid chromatography with ultraviolet detection. Journal of Chromatography B. 864(1-2): 161- 167(2008).
16. M. Gandhimathi, M. Sarvanakumar, T.K.Ravi. Validated Ion Pair HPLC method for simultaneous estimation of ceftriaxone sodium and tazobactam sodium in dosage form.International Journal of Pharma and Bio Sciences. 1(4): 17-22(2010).
17. V. Augey, P.Y. Grosse, G. Albert, M. Audran, F. Bressolle. Highperformance liquid chromatographic determination of tazobactam and piperacillin in human plasma and urine. Journal of Chromatography B: Biomedical Sciences and Applications. 682(1): 125-136(1996).
18. Sharma, S., & Sharma, M. C. (2011).Simultaneous Estimation and Validation of Ceftriaxone Sodium and Tazobactum Sodium from Pharmaceutical Dosage using Indigo carmine, Methyl orange dye. World Journal of Chemistry, 6(1), 53-58
19. Narendra Kumar, R., Rao, G. N., & Naidu, P. Y. (2010).Stability indicating fast LC Method for determination of Ceftriaxone and Tazobactam for Injection Related Substances in Bulk and Pharmaceuticals formulation. International Journal of Applied Biology and Pharmaceutical Technology, 1(1), 145-157.
20. Liu, Y., & Yang, S. X. (2005).Determination of ceftriaxone sodium/tazobactam sodium for injection by HPLC. Chinese Pharmaceutical Journal, 40(20), 1584-1586.
21. Panchal, V. J., Desai, H. T., & Dave, A. G. (2014). Development and Validation of Stability Indicating Method for Simultaneous Estimation of Ceftriaxone and Tazobactam Injection using RP-UPLC Method. International Journal of Pharmaceutical Sciences and Research, 5(6), 2398-2405.
22. A. LakshmanaRao, K. Sai Krishna, Ch. Kiran Kumar and T. Raja. Simultaneous determination of pipercillin and tazobactam in bulk and pharmaceutical dosage forms by RPHPLC. international Journal of Pharmacy and Pharmaceutical Sciences. 3(2): 134-136(2011).
23. Sutherland, Christina A., and David P. Nicolau."Development of an HPLC method for the determination of ceftolozane/tazobactam in biological and aqueous matrixes." Journal of chromatographic science 54.6 (2016): 1037-1040.