Research Article

Development and Validation of a Stability-Indicating RP-HPLC Method for the Simultaneous Estimation of Ceftolozane and Tazobactam in Pharmaceutical Dosage Form

Bhavesh Mali*, Kamal Singh Rathore, Anju Goyal
Department of Pharmaceutical Quality Assurance, Bhupal Nobles' College of Pharmacy, Bhupal Nobles' University, Udaipur (Rajasthan) 313001, India.
***Corresponding author's E-mail:** bhaveshmali5678@gmail.com

Received: 06-05-2026; **Revised:** 23-07-2026; **Accepted:** 30-07-2026; **Published online:** 20-08-2026.

ABSTRACT

A new method was established for simultaneous estimation of Ceftolozane and Tazobactam by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Ceftolozane and Tazobactam by using Inertsil ODS 4.6*250mm, 5µ, flow rate was 1 ml per min, detection wavelength was 220 nm. The instrument used Waters UPLC with auto sampler and PDA detector, Empower-software version-2. The acceptance criteria of precision is RSD should be not more than 2.0% and the method shows precision 0.7 and 0.1 for Ceftolozane and Tazobactam which shows that the method is precise. The linearity of Ceftolozane and Tazobactam was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The accuracy limit is the percentage recovery should be in the range of 98.0% - 102.0%. The total recovery was found to be 99.40% and 99.74% for Ceftolozane and Tazobactam. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The acceptance criteria for LOD and LOQ are 3 and 10. The LOD and LOQ for Ceftolozane were found to be 3.00 and 9.98 and LOD and LOQ for Tazobactam were found to be 2.98 and 10.00. The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method has good system suitability and precision under given set of conditions. Hence the suggested method can be used for routine analysis of Ceftolozane and Tazobactam in API and Pharmaceutical dosage form.

Keywords: Ceftolozane and Tazobactam, Inertsil ODS, LOQ, LOD, method validation.

INTRODUCTION

Ceftolozane is structurally characterized by a β-lactam ring fused to a dihydrothiazine ring, typical of cephalosporins, with side chains that enhance its stability against many β-lactamases. In pharmaceutical dosage forms, it is usually formulated as ceftolozane sulfate and commonly combined with tazobactam, a β-lactamase inhibitor, to extend its antibacterial spectrum. The drug is mainly administered via the parenteral route, as it shows poor stability and limited absorption in the gastrointestinal tract.[1-5]

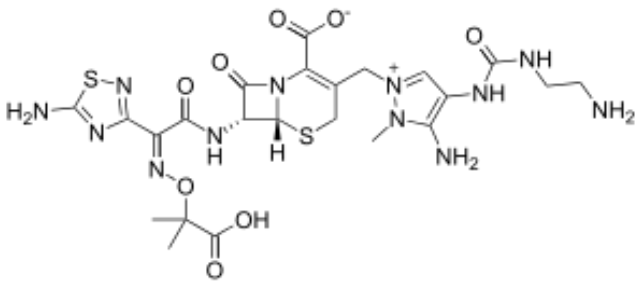

Figure 1: Structure of Ceftolozane

IUPAC Name: 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**.**

Mol Formula: $C_{23}H_{30}N_{12}O_8S_2$

Molecular Weight: 666.689

Category: Beta lactum inhibitor.

Solubility: Soluble in water, alcohol, and in methylene.

Melting Point: 180 -223 °C

pKa: 2.49

Protein Binding: 91%

Metabolism: Negligible.

Half Life: 3 hr

Mechanism of Action:

Ceftolozane belongs to the cephalosporin class of antibacterial drugs. The bactericidal action of ceftolozane results from inhibition of cell wall biosynthesis, and is mediated through binding to penicillin-binding proteins (PBPs). Ceftolozane is an inhibitor of PBPs of *P. aeruginosa* (e.g., PBP1b, PBP1c, and PBP3) and *E. coli* (e.g., PBP3).[6-10]

Tazobactum

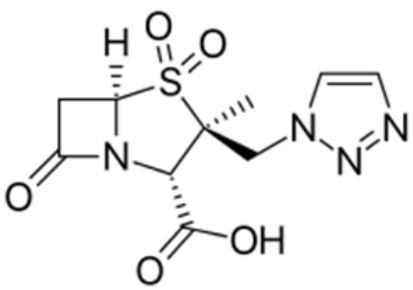

Figure 2: Structure of Tazobactum

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

Tazobactam is a β-lactamase inhibitor structurally related to penicillanic acid sulfone, widely used in combination with β-lactam antibiotics such as ceftolozane to enhance antibacterial efficacy. Unlike antibiotics, it possesses minimal intrinsic antibacterial activity, but plays a crucial role in protecting β-lactam drugs from enzymatic degradation.[11-14]

IUPAC Name: (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.

Molecular Formula : $C_{10}H_{12}N_4O_5S$

Molecular Weight :300.289

Category : Beta lactum Inhibitor.

Solubility : Soluble in water

Melting Point : 120-185°C

pKa : 2.86

Protein Binding : 100% (intravenous)

Metabolism : Nil

Mechanism of Action:

Tazobactam broadens the spectrum of piperacillin by making it effective against organisms that express beta-lactamase and would normally degrade piperacillin. Tazobactam acts by irreversibly inhibiting β-lactamase enzymes produced by resistant bacteria. It binds to the active site of these enzymes and forms a stable acyl-enzyme complex, thereby preventing the hydrolysis of the β-lactam ring of companion antibiotics. This inhibition restores the activity of drugs like ceftolozane against β-lactamase-producing organisms. Its action is particularly effective against Class A β-lactamases, though it has limited activity against some Class B and Class D enzymes.[15-19]

MATERIALS AND METHODS

Table 1: List of Instruments used

S.No	Instrument	Soft ware	Model	Company
1	HPLC	software: Empower 2,	2695 separation module, UV detector.	WATERS
2	UV/VIS spectrophotometer	UV-vin 5	UV 3000⁺	LABINDIA
3	pH meter	N/A	AD 1020	Adwa
4	Weighing machine	N/A	ER-200A	Afcoset

Optimization of Column:

The method was performed with various columns like C18 column Phenomenexcolumn, YMC, and Inertsil ODS column. Inertsil ODS (4.6 x 250mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1.0 ml/min flow.

METHOD DEVELOPMENT TRAILS

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used: Waters UPLC with auto sampler and PDA detector.

Temperature: Ambient (25° C)

Mode of separation: Isocratic mode

Column: Inertsil ODS 4.6*250mm, 5µ

Buffer: 0.1% Orthophosphoric acid

pH: 3.0

Mobile phase: 50% buffer 50% Methanol

Flow rate: 1 ml per min

Wavelength: 220 nm

Injection volume: 20 µl

Run time: 10 min.

PREPARATION OF BUFFER AND MOBILE PHASE:

Preparation of 0.1% OPA buffer:

1 ml of OPA is taken in 1000 ml of HPLC water pH was adjusted with 0.1M NAOH up to 3.0. Final solution was filtered through 0.45 µm Membrane filter and sonicate it for 10 mins.

Preparation of mobile phase:

Accurately measured 500 ml (50%) of above buffer and 500 ml of Methanol HPLC (50%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

ASSAY:

Standard Solution Preparation:

Accurately weigh and transfer 40 mg of Ceftolozane and 20 mg of Tazobactam working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 40 mg of Ceftolozane and 20 mg of Tazobactam working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure: Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for the Ceftolozane & Tazobactam peaks and calculate the %Assay by using the formulae.

$$\%\ Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

DEGRADATION STUDIES:

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Ceftolozane and Tazobactam using the proposed method.

Preparation of stock:

Accurately weigh and transfer 40 mg of Ceftolozane and 20 mg of Tazobactam working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Hydrolytic degradation under acidic condition

Pipette 0.3 ml of above solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.44 microns syringe filters and place in vials.

Hydrolytic degradation under alkaline conditions

Pipette 0.3 ml of above solution into a 10ml volumetric and add 3ml of 0.1N NaOH was added in 10ml of volumetric flask. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent. Filter the solution with 0.44 microns syringe filters and place in vials.

Thermal induced degradation

Ceftolozane and Tazobactame sample was taken in petridish and kept in Hot air oven at 110^0 C for 3 hours. Then the sample was taken and diluted with diluents and injected into UPLC and analyzed.

Oxidative degradation

Pipette 0.3 ml above stock solution into a 10ml volumetric flask and 1ml of 30% w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials.

Photo degradation:

Pipette 0.3 ml above stock solution into a 10ml volumetric flask and expose to sunlight for 24hrs and the volume was made up to the mark with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

RESULTS AND DISCUSSION

Table 2: Results of System Suitability Parameters

S.No.	Name	RT(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Ceftolozane	1.867	191234	248716		1.18	3828.18
2	Tazobactam	2.812	107018	28167	5.06	1.11	3202.01

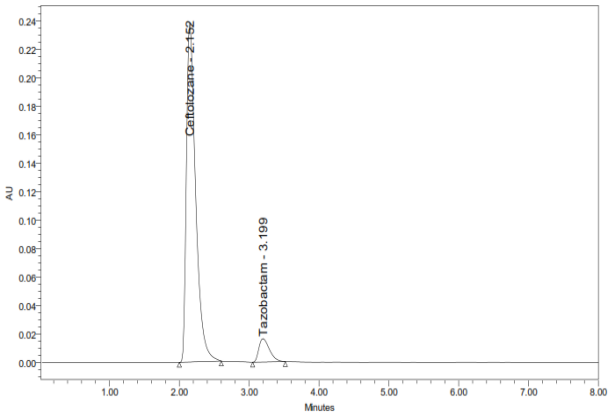

Figure 3: Chromatogram for System Suitability

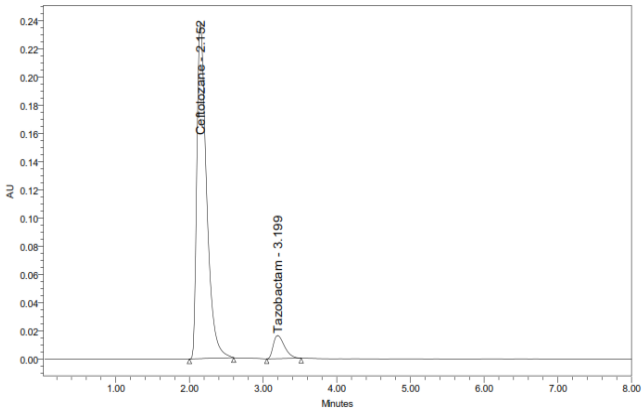

Figure 4: Chromatogram for Standard

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

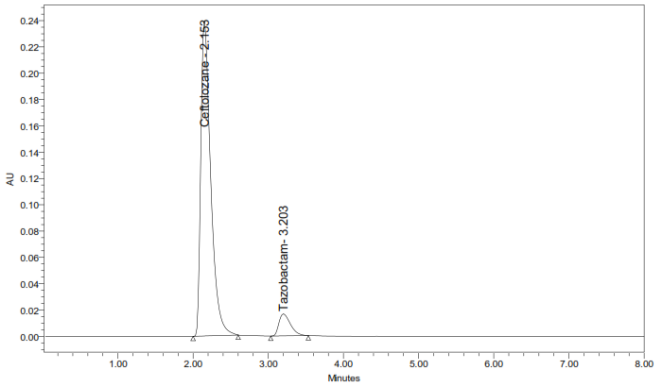

Figure 5: Chromatogram for Sample

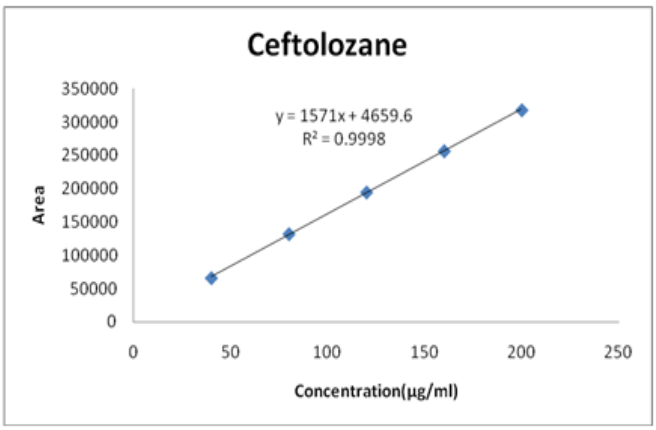

Figure 6: Calibration graph for Ceftolozane

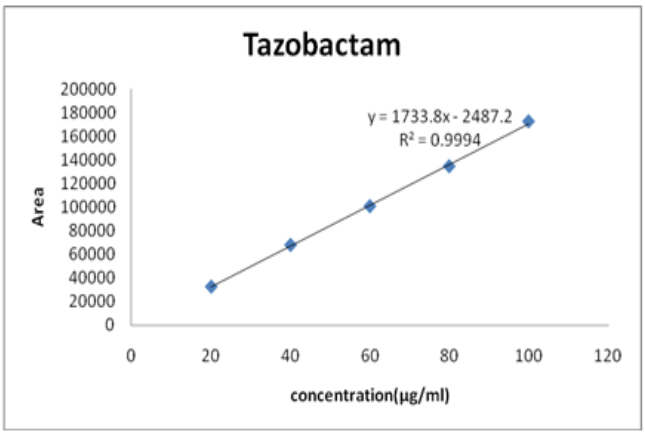

Figure 7: Calibration graph for Tazobactam

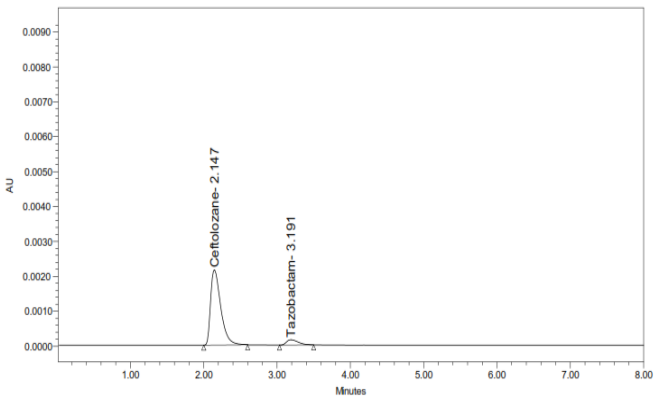

Figure 8: Chromatogram for LOD

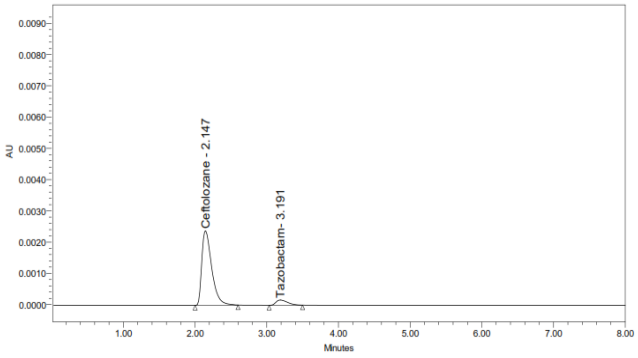

Figure 9: Chromatogram for LOQ

Table 3: Analytical Performance Parameters of Ceftolozane and Tazobactam

Parameters	Ceftolozane	Tazobactam
Slope (m)	1571	1733.8
Intercept (c)	4659.6	2487.2
Correlation coefficient (R^2)	0.999	0.999

Table 4: Results of Precision for Ceftolozane

Injection	Area
Injection-1	191345
Injection-2	191232
Injection-3	191671
Injection-4	191999
Injection-5	192898
Injection-6	194679
Average	192304.0
Standard Deviation	1308.1
%RSD	0.7

Table 5: Results of Precision for Tazobactam

Injection	Area
Injection-1	107339
Injection-2	107232
Injection-3	107131
Injection-4	107399
Injection-5	107018
Injection-6	107089
Average	107201.3
Standard Deviation	148.4
%RSD	0.1

Table 6: Results of Intermediate Precision for Ceftolozane

Injection	Area
Injection-1	192345
Injection-2	192432
Injection-3	192971
Injection-4	192899
Injection-5	192898
Injection-6	192333
Average	192646.3
Standard Deviation	305.8
%RSD	0.2

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

Table 7: Results of Intermediate precision for Tazobactam

Injection	Area
Injection-1	104533
Injection-2	104232
Injection-3	104531
Injection-4	104399
Injection-5	104018
Injection-6	104689
Average	104400.3
Standard Deviation	241.9
%RSD	0.2

Table 8: Results of LOD

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Ceftolozane	58	174	3.00
Tazobactam	58	173	2.98

Table 9: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Ceftolozane	58	579	9.98
Tazobactam	58	580	10.00

Table 10: Degradation Results for Ceftolozane and Tazobactam

Sample Name	Ceftolozane		Tazobactam	
	Area	% Degraded	Area	% Degraded
Standard	191642	100	107223	100
Acid	183252	4.38	98959	7.71
Base	183532	4.23	98921	7.74
Peroxide	183253	4.38	98978	7.69
Thermal	187552	2.13	98851	7.81
Photo	186452	2.71	98789	7.87

CONCLUSION

The current research work demonstrates the successful development and validation of an HPLC method for the analysis of Ceftolozane and Tazobactam. Multiple trials were conducted with varying chromatographic conditions to determine the optimal parameters. Inertsil ODS 4.6*250mm, 5µ, flowrate was1 ml per min, detection wavelength was 220nm.TheinstrumentusedWaters UPLC with auto sampler and PDA detector, Empower-softwareversion-2. The acceptance criteria of precision is RSD should be no more than 2.0% and the method show precision 0.7 and 0.1 for Ceftolozane and Tazobactam which shows that the method is precise. The linearity of Ceftolozane and Tazobactam was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The accuracy limit is the percentage recovery should be in the range of 98.0% - 102.0%. The total recovery was found to be 99.40% and 99.74% for Ceftolozane and Tazobactam. The acceptance criteria of intermediate precision is RSD should be no more than 2.0% and the method shows precision 0.2 and 0.2 for Ceftolozane and Tazobactam which shows that the method is repeatable when performed in different days also. The acceptance criteria for LOD and LOQ is 3 and 10. The LOD and LOQ for Ceftolozane were found to be 3.00 and 9.98 and LOD and LOQ for Tazobactam was found to be 2.98 and 10.00 the values, demonstrating the method's capability to detect and quantify low concentrations of the analytes. The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions**.**

In conclusion, the optimized HPLC method was proven to be reliable and effective for the simultaneous determination of Ceftolozane and Tazobactam. It meets all regulatory requirements for system suitability and validation parameters, ensuring its applicability for routine analysis in pharmaceutical quality control. This validated method provides a robust tool for ensuring the quality and efficacy of these critical therapeutic compounds.

REFERENCES

1. Becket and Stenlake, Practical pharmaceutical chemistry, part 24th edition CBS publications and distributors, 2005.
2. P.D. Sethi, HPLC quantitative analysis of pharmaceutical formulations CBS publications and distributors, 1st edition, 2001.
3. B.K Sharma, instrumental method of chemical analysis, 23rd edition, goal publishers 2004.
4. Practical HPLC method development Lloyd R.Snyder, Joseph J. Kirkland, Joseph L. Glajch, second edition.
5. Swarbrick James, C. Boylan James, Encyclopedia of pharmaceutical technology, Vol. I (1998) 217-224.
6. Lindsay Sandy, HPLC by open learning, (1991) 30-45.
7. W. J. Lough, I. W. W. Wainer, HPLC fundamental principles and practices, (1991) 52-67.
8. http://hplc.chem.shu.edu/NEW/HPLC_Book/
9. Szepei Gabor., HPLC in pharmaceutical Analysis, Vol. I (1990) 101-173.
10. G. H. Jeffery, J. Bassett, Vogel's textbook of Quantitative Chemical Analysis, 5th Ed (1991) 217-235.
11. H. Willard Hobart, L. L. Merritt, A. Dean John, Instrumental Methods of Analysis, 7th Ed, CBS Publishers, 580-610.
12. B.K. Sharma, Instrumental Methods of Chemical Analysis, 20th Ed (2001) 54-83.
13. .R. Meyer Veronica, Practical High Performance Liquid Chromatography, 2nd Ed (1993) 26, 27, 40, 222, 246 and 258.
14. Validating chromatographic methods, David M.Bliesner.

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

15. International conference on harmonization**:** ICH Q 2 (R1) Validation of Analytical Procedures: Text and Methodology 1995.

16. Sherif Abdel-Naby Abdel-Gawad, Abdullah Alyalkahtani2\ andelsadig Hassan Khamis, Simultaneous Quantification of Ceftolozane and Tazobactam by Reversed Phase – HPLC in Pure and Dosage Forms, Ijbpas, October, 2015;4(10): 6211-6219.

17. Pinak Patel,Vidisha Patel, GautamChauhan, Development and Validation of High Performance Thin Layer Chromatographic Method for Simultaneous Estimation of Ceftriaxone Sodium and Tazobactam Sodium in Their Combined Pharmaceutical Formulation. International Journal of Pharmacy and Pharmaceutical Sciences, 2014;6(5):26-32.

18. Sutherland CA, NicolauDP. Development Of an HPLC Method for the Determination of Ceftolozane/Tazobactam In Biological and Aqueous Matrixes, J Chromatogr Sci. 2016 Jul;54(6):1037-40.

19. K.Neelima, R.Nikhila, S.Selina, N.Appalraju, Y.Rajendra Prasad, Analytical Method Development and Validation of Cefepime Hydrochloride and Tazobactam Sodium in Bulk and Sterile Dry Powder for Injection by Gradient Rp-Hplc, Iajpr. 2013; 3(10): 8044-8051.

For any questions related to this article, please reach us at: globalresearchonline@rediffmail.com

New manuscripts for publication can be submitted at: submit@globalresearchonline.net and submit_ijpsrr@rediffmail.com

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.