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A new RP-HPLC Stability Indicating Method Developed and Validated for the Simultaneous Estimation of Ceftolozane and Tazobactum in Pharmaceutical Dosage Form

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A b s t r a c t

The aim of this study is to develop and validate a new RP-HPLC method for the Simultaneous Estimation of Ceftolozane and Tazobactum in Pharmaceutical Dosage Form. The estimation of Tazobactum and Ceftolozone was done by RP-HPLC. The assay of Tazobactum and Ceftolozone was performed with tablets and the % assay was found to be 99.72 and 99.80 which shows that the method is useful for routine analysis. The linearity of Tazobactum and Ceftolozone 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 acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.60 and 0.30 for Tazobactum and Ceftolozone which shows that the method is precise. The acceptance criteria for LOD and LOQ is 3 and 10. The LOD and LOQ for Tazobactum was found to be 3.02 and 10.00 and LOD and LOQ for Ceftolozone was found to be 3.00and 9.98. 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.

Keywords: Ceftolozane, Tazobactum, LOD and LOQ, RP-HPLC, robustness

A r t i c l e I n f o

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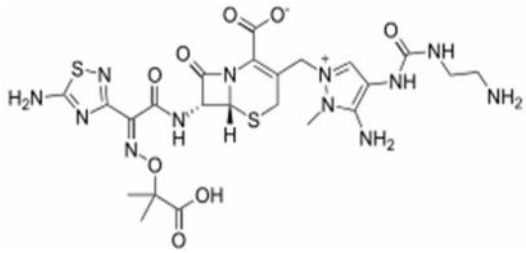
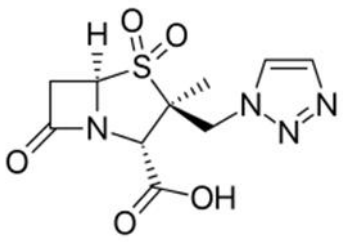
1. Introduction

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.
Molecular Formula	$C_{19}H_{22}BrNO_4S_2$
Molecular Weight	666.689 gm/mole
pKa	2.49
Solubility	Soluble in water, alcohol, and in methylene.
Category	Beta lactum inhibitor

Tazobactum



IUPAC Name	**4-(1-hydroxy-2-{[6-(4-phenylbutoxy)hexyl]amino}ethyl)-2-(hydroxymethyl)phenol**
Molecular Formula	$C_{10}H_{12}N_4O_5S$
Molecular Weight	300.289 gm/mole
pKa	2.86
Solubility	Soluble in water
Category	Beta lactum Inhibitor

2. Methodology

Table 1. Instruments used

S.No	Instrument	Model
1	HPLC	WATERS, software: Empower, 2695 separation module, PDA detector.
2	UV/VIS spectrophotometer	LABINDIA UV 3000$^+$
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

Table 2. Chemicals used

S.No	Chemical	Company Name
1	Ceftlozane	PHARMATRAIN
2	Tazobaactam	PHARMATRAIN
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	MOLYCHEM
5	Ortho phosphoric Acid	MERCK

Preparation of the ceftlozane & tazobaactam standard & sample solution:

Standard Solution Preparation:

Accurately weigh and transfer 100 mg of Ceftlozane and 50 mg of Tazobaactam 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 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh and transfer 100 mg of Ceftlozane and 50 mg of Tazobaactam 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 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents

Procedure:

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Ceftlozane and Tazobaactam peaks and calculate the %Assay by using the formulae.

System suitability:

Tailing factor for the peaks due to Ceftlozane and Tazobaactam in Standard solution should not be more than 2.0. Theoretical plates for the Ceftlozane and Tazobaactam peaks in Standard solution should not be less than 2000.

Resolution for the Ceftlozanee and Tazobaactam peaks in standard solution should not be less than 2.

Mobile Phase Optimization:

Initially the mobile phase tried was methanol: Ammonium acetate buffer and Methanol: phosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to orthophosphoric acid with buffer (pH 3), Acetonitrile in proportion 50: 50 v/v respectively.

Optimized chromatographic conditions:

Instrument used: Waters HPLC with auto sampler and 2487 UV detector

Temperature : Ambient
Column : Thermosil (4.6*100mm, 5µm)
Buffer : 1ml of orthophosphoric acid in 1000ml water, pH adjusted with NaOH.
pH : 3
Mobile phase : 50% buffer 50% Acetonitrile
Flow rate : 1 ml per min
Wavelength : 220 nm
Injection volume: 20 µl
Run time : 10 min.

Linearity

Preparation of stock solution:

Accurately weigh and transfer 100 mg of Ceftlozane and 50 mg of Tazobaactam 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)

Procedure:

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. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

3. PRECISION:

Preparation of stock solution:

Accurately weigh and transfer 100 mg of Ceftlozane and 50 mg of Tazobaactam 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 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents

Procedure:

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 to be within the specified limits.

Accuracy:

For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same.

Preparation of Standard stock solution:

Accurately weigh and transfer 100 mg of Ceftlozane and 50 mg of Tazobaactam working standard into a 10 ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):

Accurately weigh and transfer 50 mg of Ceftlozane and 25 mg of Tazobaactam 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 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents

3. Results and Discussion

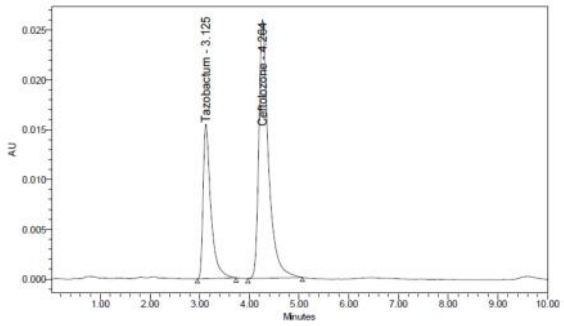


Figure 1: Chromatogram for system suitability

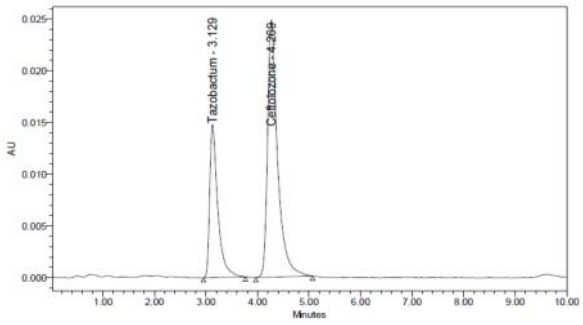


Figure 2: Chromatogram for Standard

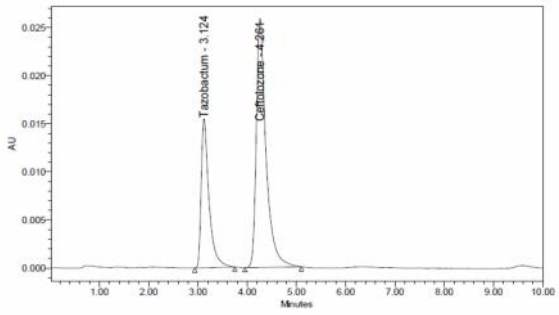


Figure 3: Chromatogram for Sample

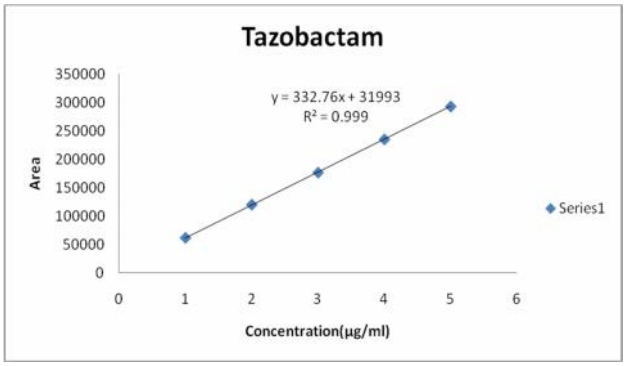


Figure 4: Calibration graph for Tazobactum

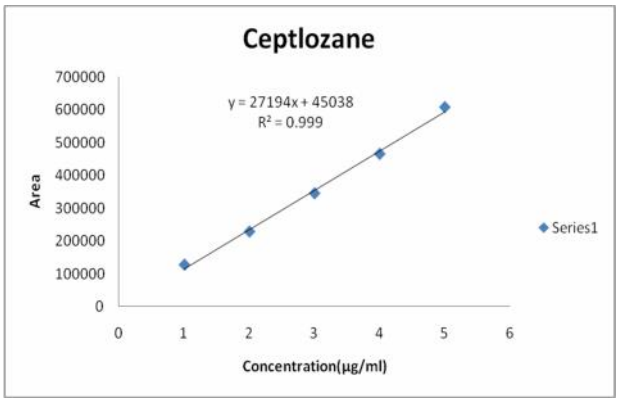


Figure 5: Calibration graph for Ceftolozone

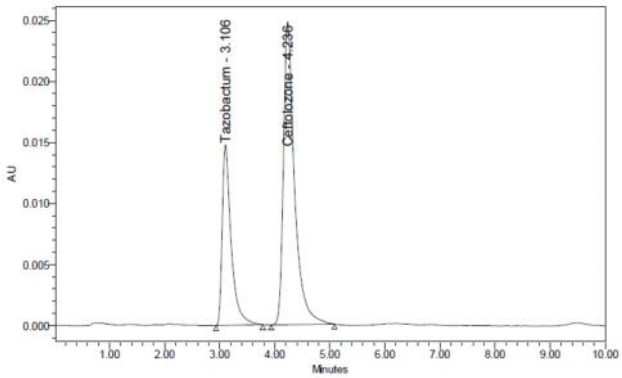


Figure 6: Chromatogram for Precision -6

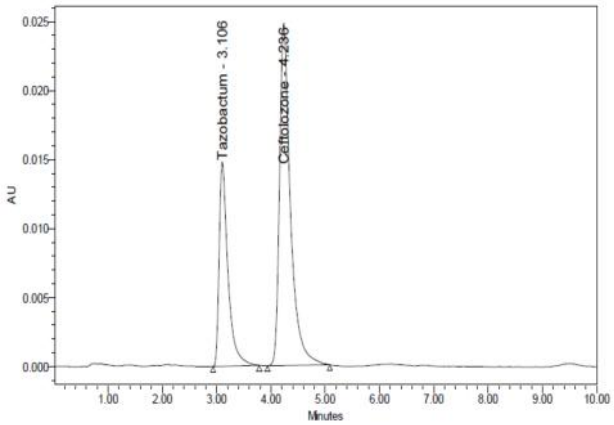


Figure 7: Chromatogram for ID Precision -5

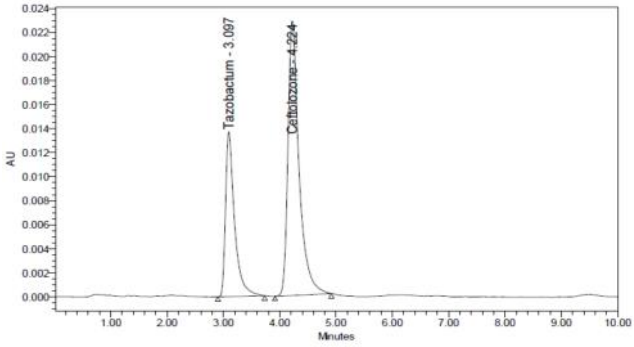


Figure 8: Chromatogram for Accuracy 100%-3

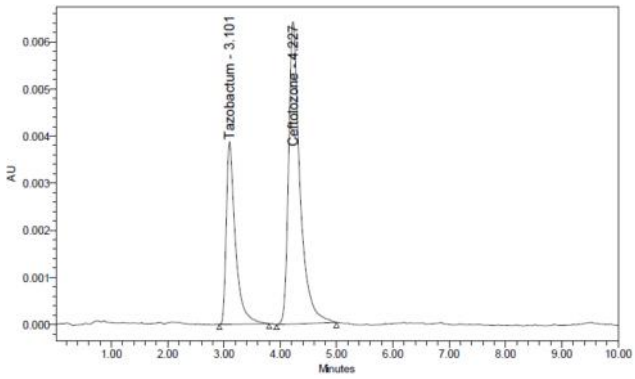


Figure 9: Chromatogram for Accuracy 150%-3

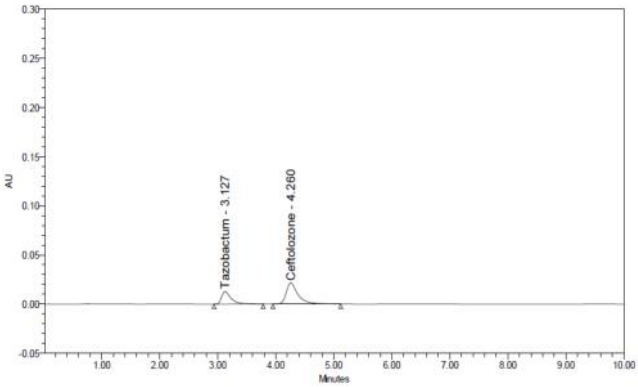


Figure 10: Chromatogram of Tazobactum, Ceftolozone showing LOD

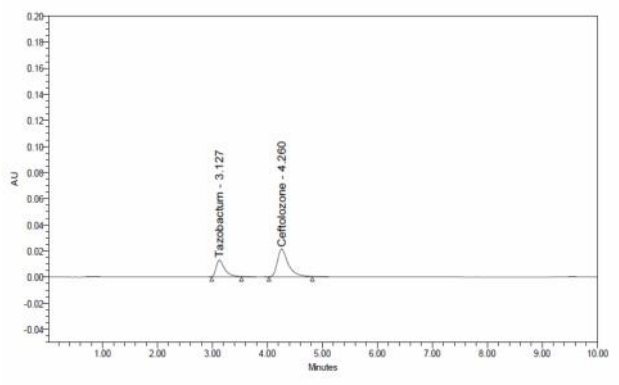


Figure 11: Chromatogram of Tazobactum, Ceftolozone showing LOQ

Table 3: Results for variation in flow for Tazobactum

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	2025.5	1.18
2	1.0	3961.26	1.15
3	1.1	2644.17	1.13

Table 4: Results for variation in flow for Ceftolozone

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	2452	1.12
2	1.0	2718.66	1.64
3	1.1	2255	1.22

*****Results for actual flow (1.5ml/min) have been considered from Assay standard**.**

Table 5: Degradation results

Sample Name	TAZO		CEP	
	Area	% Degraded	Area	% Degraded
Standard	171146.0	9.27	346468.0	6.07
Acid	155289	8.02	325453	5.37
Base	157420	4.72	327849	6.16
Peroxide	163076	4.35	325131	5.23
Thermal	163704	8.37	328347	4.94
Photo	156820	9.27	329359	6.07

4. Conclusion

The estimation of Tazobactum and Ceftolozone was done by RP-HPLC. The assay of Tazobactum and Ceftolozone was performed with tablets and the % assay was found to be 99.72 and 99.80 which shows that the method is useful for routine analysis. The linearity of Tazobactum and Ceftolozone 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 acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.60 and 0.30 for Tazobactum and Ceftolozone which shows that the method is precise. The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.40 and 0.30 for Tazobactum and Ceftolozone which shows that the method is repeatable when performed in different days also. The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 100.34% and 100.01% for Tazobactum and Ceftolozone. 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 is 3 and 10. The LOD and LOQ for Tazobactum was found to be 3.02 and 10.00 and LOD and LOQ for Ceftolozone was found to be 3.00and 9.98. 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.

5. References

[1] Dr. Kealey and P.J Haines, Analytical Chemistry, 1stedition, Bios Publisher, (2002), Pp 1-7.

[2] A.Braithwait and F.J.Smith, Chromatographic Methods, 5thedition, Kluwer Academic Publisher, (1996), Pp 1-2.

[3] Andrea Weston and Phyllisr. Brown, Hplc Principle and Practice, 1st Edition, Academic Press, (1997), Pp 24-37.

[4] Yuri Kazakevich and Rosario Lobrutto, Hplc For Pharmaceutical Scientists, 1stedition, Wiley Interscience A Johnwiley & Sons, Inc., Publication, (2007), Pp 15-23.

[5] Meyer V.R. Practical High-Performance Liquid Chromatography, 4th Ed. England, John Wiley & Sons Ltd, (2004), Pp 7-8.

[6] Sahajwalla Cg A New Drug Development, Vol 141, Marcel Dekker Inc., New York, (2004), Pp 421–426.

[7] Ich Q2a, “Validation of Analytical Methods, Definitions And Terminology”, Ich Harmonized Tripartite Guideline, (1999).

[8] Introduction To Analytical Method Validation (Online), Available From: Url: Http://Www.Standardbase.Hu/Tech/Hplc%20validation%20pe.Pdf

[9] "Briefing Package: Ceftolozane and Tazobactum. Anti-Infective Drugs Advisory Committee Meeting" (Pdf). Fda.Gov. U.S. Food and Drug Administration. Retrieved 27 June 2015.

[10] Sherif Abdel-Naby Abdel-Gawad, Abdullah Alyalkahtani2andelsadig Hassan Khamis, Simultaneous Quantification of Ceftolozane and Tazobactam By Reversed Phase – Hplc In Pure And Dosage Forms, Ijbpas, October, 2015, 4(10): 6211-6219.

[11] Sutherland Ca1, Nicolau Dp2, 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.

[12] 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.

[13] Pinak Patel,Vidisha Patel1, Gautam Chauhan, 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, Vol 6, Issue 5, 2014.