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Stability indicating RP-HPLC method development and validation for the simultaneous estimation of ceftriaxone and tazobactum in sterile powder for injection

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ABSTRACT

A simple, rapid, precise and accurate method is developed for the quantitative simultaneous determination of ceftriaxone and tazobactum in bulk and pharmaceutical formulations. Separation of ceftriaxone and tazobactum was successfully achieved by using Inertsil C18 ODS column 250X4.6mm, 5µm in an isocratic mode using water and acetonitrile (80:20) at a flow rate of 1.0 ml/min and was monitored at 254 nm with a retention time of 3.049 minutes and 4.317 minutes for ceftriaxone and tazobactum respectively. The method was validated and the response was found to be linear in the drug concentration range of 20µg/ml to 80 µg/ml for ceftriaxone and 5 µg/ml to 35 µg/ml for tazobactum. The values of the correlation coefficient were found to be 0.999 for ceftriaxone and 0.999 for tazobactum respectively. The LOD and LOQ for ceftriaxone were found to be 0.021 and 0.064 respectively. The LOD and LOQ for tazobactum were found to be 0.030 and 0.091 respectively. The percentage recovery for ceftriaxone and tazobactum were found to be 98-102% respectively which indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard with the sample. The method was extensively validated according to ICH guidelines for Linearity, Accuracy, Precision, Specificity and Robustness. Stability of the drugs was determined by using acid/base, thermal, oxidative stress testing.

Keywords: Ceftriaxone; Tazobactum; RP-HPLC.

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INTRODUCTION

Analytical method development and validation places an important role in drug discovery and manufacture of pharmaceuticals[1]. Development of simple and reproducible analytical methods for estimation of multi component drugs is very important part of quality control and for social awareness which is established in present work[2].

Ceftriaxone[3] is a beta-lactum third generation broad-spectrum cephalosporin antibiotic. Ceftriaxone works by inhibiting the mucopeptide synthesis in the bacterial cell wall. The beta-lactam moiety of Ceftriaxone binds to carboxypeptidases, endopeptidases and transpeptidases in the bacterial cytoplasmic membrane.

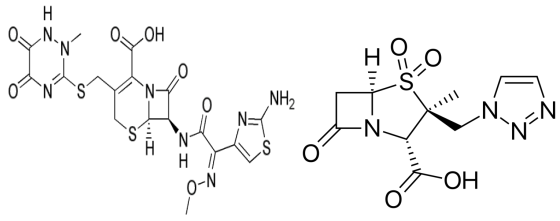

Figure 1: Ceftriaxone and Tazobactum

Tazobactum[4] broadens the spectrum of piperacillin by making it effective against organisms that express beta-lactamase and would normally degrade

piperacillin**.** Tazobactum is a compound which inhibits the action of bacterial beta-lactamases. It is added to the extended spectrum beta-lactam antibiotic piperacillin**.**

MATERIALS AND METHODS

Chemicals and Reagents: Ceftriaxone and Tazobactum were kindly gifted by Nutech Biosciences Pvt Ltd, Hyderabad certified to contain 99.8% and 99.9% purity respectively. The drugs were used without further purification. All the solvents used in analysis were of HPLC grade. Tazox injections (label claim 1000 mg of Ceftriaxone and 125 mg Tazobactum) was used in analysis.

HPLC Method

Instrument: LC system used consists of Waters 2690 pump model with universal loop injector of injection capacity 20 µL. Detector consists of dual wavelength photo diode detector. The column used was Inertsil C18 Column, 5µ (250× 4.6 mm) at ambient temperature. Different mobile phases were tested in order to find the best conditions, for separating both the drugs simultaneously.

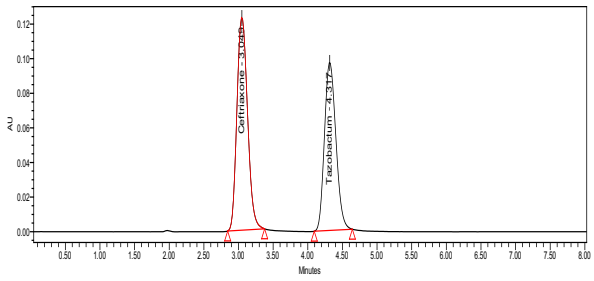

Figure 2: Chromatogram of Standard solution of ceftriaxone and Tazobactum

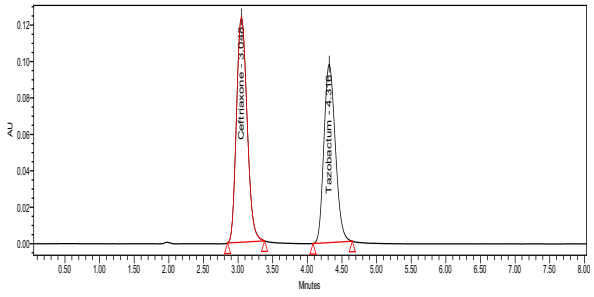

Figure 3: Chromatogram of Sample solution of ceftriaxone and Tazobactum

Optimized Chromatographic conditions: The mobile phase consisting of Water: Acetonitrile (80:20 v/v) was selected because it was found that it ideally resolve the peaks with retention time (RT) 3.04 min and 4.31 min for Ceftriaxone and Tazobactum respectively. Wavelength was selected by scanning all standard drugs over a wide range of wavelength 200nm to 350nm. Both the components showed reasonably good response at 254 nm.

Standard Preparation: Weigh accurately and transfer 10 mg of ceftriaxone and 1.25 mg of tazobactum in a 10ml volumetric flask, dissolved with 5ml of mobile phase and sonicate for 10 min and finally made up the volume with the mobile phase. From this solution 0.4 ml was transferred in a 10 ml volumetric flask and the volume was made up with the mobile phase to get 40 ug/ml solution of ceftriaxone and 5 ug/ml solution of Tazobactum. This solution was filtered through a 0.45 um filter before use.

Sample Preparation: Accurately weighed 56.25 mg (eq.wt) of sample was transferred to a 10 ml volumetric flask, dissolved with 5 ml of mobile phase and sonicated for 10 min and finally made up the volume with the mobile phase. The solution was filtered through a Whatman filter paper and from the filtrate, 0.4 ml was transferred in a 10 ml volumetric flask and the volume was made up with the mobile phase. This solution was filtered again through a 0.45 um filter before use.

Recovery studies: To check the accuracy of sample by the developed methods and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method at 80, 100 and 120% level. From the total amount of drug found, the percentage recovery was calculated. The results are reported in Table 1.

Analysis data of Capsule formulations

Table 1: Analysis data of Capsule formulations

Parameter	Hplc	
	Ceftriaxone	Tazobactum
Label claim(mg)	1000	125
Drug found	1002.7	125.4
% Accuracy	99-101	99-101
% Recovery±RSD	100.27±0.45	100.38±0.36

RESULTS AND DISCUSSION

Preparation of Calibration Curves by HPLC: In a series of 10 ml volumetric flask several dilutions of Ceftriaxone (20-80µg/ml) and Tazobactum (5-35 µg/ml) were prepared using mobile phase as solvent. Each solution was injected into HPLC system and the chromatograms were recorded. The peak areas of both drugs were calculated and the respective calibration curves were plotted against ratio of area under curve and concentration of drug.

The equations of the regression lines obtained are for Ceftriaxone: R^2 = 0.999 For Tazobactum: R^2 = 0.998

HPLC Method Validation: As per the ICH guidelines, the method validation parameters checked were linearity, accuracy, Specificity, precision, limit of detection, limit of quantitation.

Linearity: A Series of solutions are prepared using Ceftriaxone and Tazobactum working standards at concentration levels from 20ppm to 80ppm of

ceftriaxone and 5ppm to 35ppm of tazobactum of target concentration. Inject into the chromatographic system and Measure the peak area.

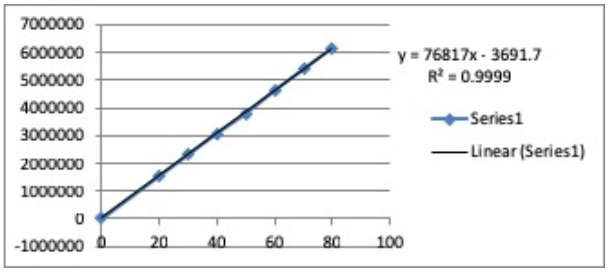

Figure 4: Linearity Curve of Ceftriaxone

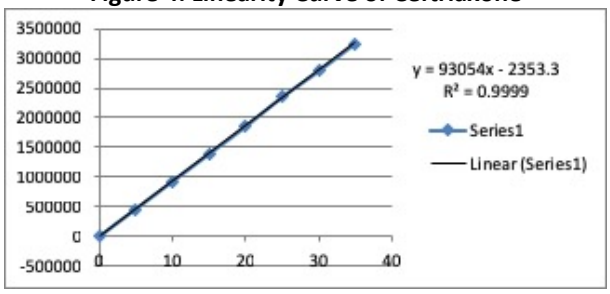

Figure 5: Linearity Curve of Ceftriaxone

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

Precision*:* Precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. Precision was demonstrated by repeatability and intermediate precision measurements of peak area and peak symmetry parameters of HPLC method for each title ingredients. The repeatability (within-day in triplicates) and intermediate precision (for 2 days) were carried out at five concentration levels for each compound. Triplicate injections were made and the obtained results within and between the days of trials were in acceptable range. The value of %RSD for Ceftriaxone and Tazobactum were found to be less than 2 indicates that the developed method is precise.

Accuracy: Accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method should be established across its linearity range. Accuracy was performed in three different levels, each level in triplicate for Ceftriaxone and Tazobactum using standards at 80%, 100% and 120%. Each sample was analysed in triplicate for each level. The mean recoveries were found in the range of 98 – 102 %, by which we can say the method was accurate.

limit of detection (LOD) and limit of quantitation (LOQ): It is calculated according to ICH recommendations where the approach is based on the signal to noise ratio. Chromatogram signals obtained with known low concentrations of analytes were compared with the signals of blank samples. A signal-to-noise ratio 3:1 and 10:1 was considered for calculating LOD and LOQ respectively.

Table 2: Validation Parameters

Validation Parameters		HPLC	
		Ceftriaxone	Tazobactum
Calibration Range (µg mL^{-1})		20-80	5-35
Linearity Coefficient (R^2)		0.999	0.999
Precision (%RSD)	Inter Day	0.021	0.009
	Intra Day	0.018	0.023
LOD		0.021	0.030
LOQ		0.064	0.091
Tailing Factor		1.146	1.096
Theoretical Plates		10038	8358

Specifity: Volume of 20 µL of working placebo sample solution was injected into the chromatograph and the chromatogram was recorded and presented below.

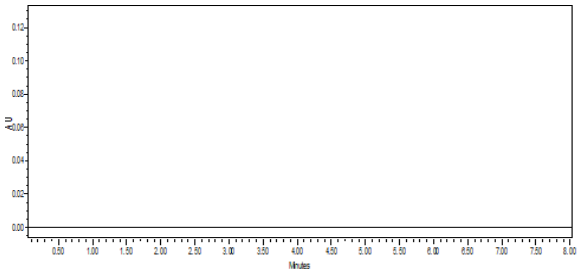

Figure 6: Chromatogram of Blank

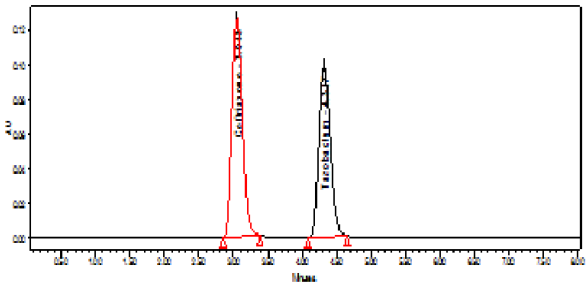

Figure 7: Chromatogram of Sample

Forced degradation (stability)

Acid/ Base Stress Testing*:* Acid/Base stress testing is performed to force the degradation of a drug substance to its primary degradation products by exposure to acidic and basic conditions over time. Acid testing was done by taking samples and was treated separately with 5.0ml of 1.0N Hydrochloric acid and kept on bench top for 10 minutes. The treated sample was analysed and tabulated. For base degradation study, sample was treated separately

with 5ml of 0.05N Sodium hydroxide and kept on bench top for 5 minutes.

Thermal Stress Testing: The thermal stress study was carried out with the sample solution at 80°C for 12 hours. Then the sample was screened for degradation products by the developed HPLC method.

Oxidative Stress Testing: Oxidative studies were performed by taking H_2O_2; tablets sample was treated separately with 5ml of 3.0%v/v solution of hydrogen peroxide and kept on bench top for 60 minutes.

Table 3: Forced Degradation for Ceftriaxone and Tazobactum

Mode of degradation	% degradation	
	Ceftriaxone	Tazobactum
Acid	0.23	1.21
Base	0.46	0.08
Oxidative	0.8	0.11
Thermal	0.79	1.08

CONCLUSION

The methods described for simultaneous estimation of Ceftriaxone and Tazobactum are found to be simple, sensitive, accurate, precise, rapid and economical. Hence method could be successfully employed for routine analysis of Ceftriaxone and Tazobactum in their combined dosage form.

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