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Determination of Ceftalazone and Tazobactum in Bulk and Pharmaceutical Dosage Forms by RP-HPLC

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ABSTRACT

New stability- indicating Reverse Phase High Performance Liquid Chromatography was developed for the simultaneous determination of Ceftalazone and Tazobactum in bulk and pharmaceutical formulations. This method was accurate, precise and sensitive for the simultaneous analysis of Ceftalazone and Tazobactum. Optimization of the mobile phase can be done by using different combinations of organic solvents and buffer solutions were used on Inertsil ODS C18 (150 mm × 4.6 mm, 5 µ) column. The OPA Buffer: Acetonitrile buffer mixture of 53:47% v/v was a selected method with a flow rate of about 1.0 ml/min. The advantages of this method were shortest retention time, good shapes peaks and very lowest noise with baseline stability. The retention times of Ceftalazone and Tazobactum are 2.058 and 3.709 min respectively. Quantitative linearity of Ceftalazone and Tazobactum was obeyed in the concentration range of 25-150 and 12.5-75 µg/ml respectively. The detection and quantitation limits were found to be 0.36 µg/mL and 1.09 µg/mL (Ceftalazone); 0.06 µg/ mL and 0.19 µg/ml (Tazobactum) respectively. It represents the sensitivity of the method. The higher accuracy of our established method was found due to the highest recovery percentage. The excipients are used in the injection formulations can't interfere with the determination of the drugs by the proposed RP-HPLC method. It arises due to the absence of interfering peaks found in that chromatogram.

Keywords: Ceftalazone, Tazobactum, assay, RP-HPLC

INTRODUCTION

Ceftolozane was the analog drug of cephalosporin antibiotic and it is developed to destroy the infections caused by gram-negative bacteria. This drug exhibited most resistant character than the conventional antibiotic drugs [1]. It was trialed for ventilator-associated bacterial pneumonia, urinary tract infections and intra-abdominal infections. Ceftolozane mixed with the β-lactamase inhibitor tazobactam, which prevents ceftolozane from the degradation [2-6]. Ceftolozane-tazobactam combination was used for the curing of complicated intra-abdominal infections and urinary tract infections [7]. The chemical structure of Ceftalazone was given in Figure 1.

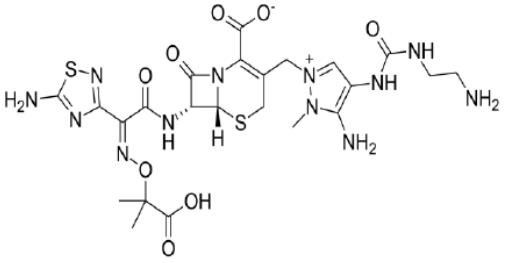


Figure 1: Chemical structure of Ceftalazone

Tazobactum drug was modification of both penicillin and sulfone and it was used as their sodium salt in medical purposes. From 1992 onwards, this drug was entered into market.

This drug mostly inhibits the both TEM and SHV-1 containing bacterial β-lactamases. The extensive combination of both Tazobactam and β-lactam antibiotic piperacillin was used to the treatment of nosocomial pneumonia. This drug in combination with piperacillin enhances the effective activity of piperacillin against the disease causing microorganisms. The chemical structure of Tazobactum was given in Figure 2.

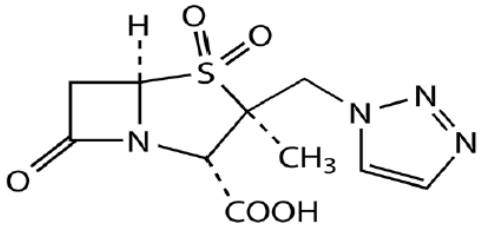


Figure 2: Chemical structure of tazobactum

The survey of literature concluded that several analytical methods were used for tazobactum [8-14] in, HPLC either individually or in combination. But the simultaneous estimation of ceftalazone and tazobactum by HPLC in both bulk drug and pharmaceutical dosage forms was not found till date. So we concentrate for the designing of one potent method for simultaneous estimation of ceftalazone and tazobactum in both bulk drug and pharmaceutical dosage forms by using Reverse phase -HPLC method.

MATERIALS AND METHODS

Chemicals and reagents

Ceftalazone and Tazobactum drug samples were obtained from Spectrum Pharma Research laboratory, Hyderabad and marketed formulation was taken from local market. Water and acetonitrile were purchased from Merck Company, Mumbai and ortho phosphate, Ortho Phosphoric acid and triethyl amine with HPLC grade are purchased from RANKEM, Mumbai.

Instrument and chromatographic conditions

In this method, 2996 Photodiode Array Detector and RP-HPLC waters 2695 separation module were equipped with other. Empower 2 software designed for data processing and data acquisition with integration of LC peak. Inertsil ODS C18, (150 × 4.6 mm, 5 µ) was used as column for the separation of analytes. Phosphate Buffer: Acetonitrile with the ration of 53:47% v/v at a flow rate of 1.0 ml/min was used as mobile phase. 0.45 µm nylon filter and ultrasonic bath were used for filtration and sonication purpose (5 min). 10 µl injection volume and 273 nm were used for sample analysis.

Preparation of phosphate buffer pH 3.6

Accurately 1 ml ortho phosphoric acid was taken in one lt volumetric flask. To this, 900 ml of milli-Q water was added and allowed to sonicate. Finally, one lt volumetric flask make up with water to the upper mark.

Preparation of solutions

Ceftalazone stock preparation (1000 µg/ml)

10 mg of Ceftalazone was accurately weighed and transferred to 10 mL of dry volumetric plask. To this, 7 mL of water: acetonitrile with 50:50 was added and allowed for sonication over 10 min and make up to the mark with diluents.

Tazobactum stock preparation (500 µg/ml)

5 mg of Tazobactum was weighed accurately and transferred into 10 ml of clean dry volumetric flask. To this, 7 mL of water: acetonitrile with 50:50 was added and allowed for sonication over 10 min and make up to the mark with diluents.

Standard preparations

Ceftalazone standard preparation (100 µg/ml)

1 mL stock solution of Ceftalazone was pipeted out into a 10 ml volumetric flask and made upto the mark with water.

Tazobactum standard preparation (50 µg/ml)

1 mL stock solution of Tazobactum was pipeted out into a 10 ml volumetric flask and made upto the mark with water.

Method validation

ICH guidelines were used to validate the particular parameters such as Precision, specificity, stability, linearity, accuracy, LOD and LOQ.

Specificity

It is one of the analytical methods to record the analyte response by the degradation products and related substances in the presence of intereferences.

Accuracy

The% recoveries of Ceftalazone and Tazobactum was used for the determination of accuracy. It was calculated by adding each analyte of known quantity to 50, 100 and 150 of three different concentration levels.

Here, six determinations were conducted at each concentration level. Finally, accuracy results were gained as a percent of recovered anlyte by this method.

Precision

It was an analytical method and generally expressed as the standard deviation. These studies were conducted by knowing the response of Ceftalazone and Tazobactum over six times. The inter-day and intra-day sample precision studies was done on the same day and on the different days for the estimation of responses of three equal concentrations. These results were replicated in the sense of relative standard deviation.

Linearity

The linear response of the entire analytical system is directly proportional to the concentration of the particular analyte. The accurate relationship towards the peak area and concentration was determined by linear regression calibration plot. Here, high significance determined from the correlation co-efficient.

Robustness

The variation of buffer composition in temperature, mobile phase and flow rate under a variety of conditions was investigated by Robustness. Theoretical plates, peak tailing and peak area does not influence the deliberate change of this method.

Limit of detection and limit of quantitation

The lowest level of analyte with measurable response was termed as LOD and least amount of analyte which has a reproducibly quantified character is known as LOQ. These parameters were determined from standard deviation response and the slope.
LOD was calculated by, LOD=3.3 × s/S
LOQ was calculated by, LOQ=10 × s/S
Where, s=Standard deviation and S=Slope of the calibration curve.

Assay of ceftalazone and tazobactum in injection

Ceftalazone and Tazobactum assays were obtained from Spectrum Pharma Research laboratory, Hyderabad and used for RP-HPLC method development. The preparation of sample solutions was done by standard protocol and these solutions were injected by HPLC injector into the system. The RP-HPLC system scanned the sample solution at 260 nm. The drugs Ceftalazone and Tazobactum were found to be estimated as 99.97% and 100.52% respectively. The chromatogram showed two separate single peaks of Ceftalazone and Tazobactum. These singlets were observed with the retention times of 2.165 and 3.929 min (Figure 3).

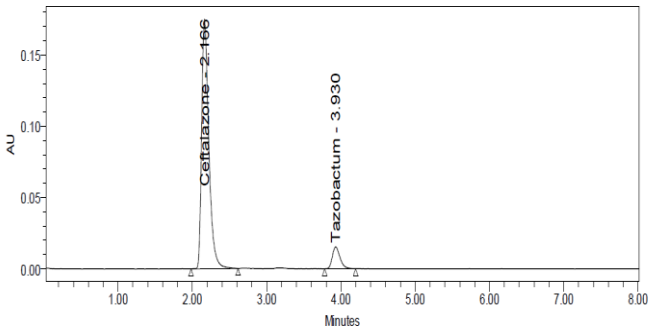


Figure 3: A typical chromatogram of Ceftalazone and Tazobactum in injection dosage form

Forced degradation studies

Stress studies are carried out by hydrolysis, oxidation, photolysis and thermal studies as per ICH guidelines.

Oxidation

1 mL of 20% hydrogen peroxide was mixed to 1 mL of stock solution of Ceftalazone and Tazobactum. These solutions allowed at 600ºC over 30 min. The resultant solution was diluted to obtain 100 and 50 µg/ml and 10 µl for the study of HPLC. These solutions were injected by HPLC injector to the instrument and consequently chromatograms were obtained. From these chromatograms stability studies of samples were discussed.

Acid degradation studies

1 mL of 2 N Hydrochloric acids was added to 1 mL of stock solution of Ceftalazone and Tazobactum and refluxed for 30 min at 60ºC. For HPLC study, the resultant solution was diluted to obtain 100 and 50 µg/ml and 10 µl. These solutions were injected by HPLC injector to the instrument and consequently chromatograms were obtained. From these chromatograms stability studies of samples were discussed.

Alkali degradation studies

1 mL of 2 N Hydrochloric acid was added to 1 mL of stock solution of Ceftalazone and Tazobactum and refluxed for 30 min at 60ºC. For HPLC study, the resultant solution was diluted to obtain 100 and 50 µg/ml and 10 µl. These solutions were injected by HPLC injector to the instrument and consequently chromatograms were obtained. From these chromatograms stability studies of samples were discussed.

Dry heat degradation studies

The standard drug solution heated at 105ºC over 6 h to analysis the dry heat degradation.

For HPLC study, the resultant solution was diluted to obtain 100 and 50 µg/ml and 10 µl. These solutions were injected by HPLC injector to the instrument and consequently chromatograms were obtained. From these chromatograms stability studies of samples were discussed.

Photo stability studies

When 1000 and 500 µg/ml of the drug solution was exposed to UV light over 7 days or 200 Watt h/m^2 in photo stability chamber then the study of photochemical stability was done.

For HPLC study, the resultant solution was diluted to obtain 100 and 50 µg/ml and 10 µl. These solutions were injected by HPLC injector to the instrument and consequently chromatograms were obtained. From these chromatograms stability studies of samples were discussed.

RESULTS AND DISCUSSION

Optimized chromatographic conditions

Different preliminary tests were compulsory to establish and validate a excellent method for the analysis of these drugs in pharmaceutical formulations. Different chromatographic conditions were employed for the analysis of the Ceftalazone and Tazobactum in both bulk and pharmaceutical dosage form. Finally the analysis was performed by using Ortho phosphoric acid: Acetonitrile in the ration of 53:47% v/v at a flow rate 1.0 ml/min. Samples were analysed at 273 nm at an injection volume of 10 µl and separation was carried by using Inertsil ODS C18, (150 × 4.6 mm, 5 µ) column. This method gave a sharp peak with less tailing for Ceftalazone and Tazobactum (Figure 4).

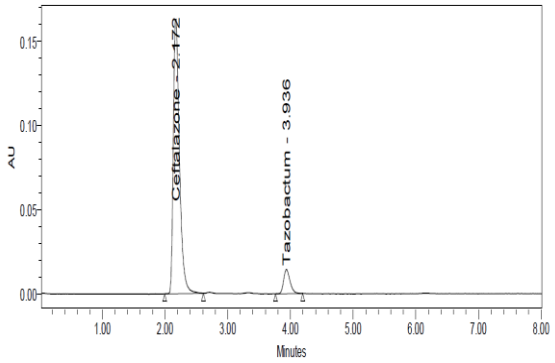


Figure 4: Standard chromatogram of Ceftalazone and Tazobactum

The specificity of the developed method including stability indicating property was performed by Forced degradation studies. Dry heat, UV light, hydrolysis, photolysis and oxidation were different conditions for carried out the degradation studies and degradation occurred in the drug substances at all conditions. Ceftalazone and Tazobactum drugs undergo acid and base hydrolysis with 2 N HCl and 2 N NaOH at 60^{o}C over a period of 30 min gave the degraded products peak with retention time of 2.637 min. Drug sample was heated with 20% H_2O_2 at 60^{o}C undergo oxidative degradation then the degraded products peaks were observed. Both Ceftalazone and Tazobactum drugs are sensitive to acid and alkali. UV light and thermal conditions cannot support the degradation of these drugs. The results of forced degradation studies were shown in Table 1.

Table 1: Results of forced degradation studies

S. No.	Injection	Ceftalazone		Tazobactum	
		% Assay	% Degradation	% Assay	% Degradation
1	Acid Degradation	96.06	3.94	96.69	3.31
2	Base Degradation	97.97	2.03	97.38	2.62
3	Peroxide	95.54	4.46	95.36	4.64
4	Thermal Degradation	99.52	0.48	98.98	1.02
5	UV Degradation	99.55	0.45	99.13	0.87
6	Neutral degradation	99.50	0.50	99.07	0.93

Ceftalazone and Tazobactum was injected over six times then recorded the corresponding peaks. So there by precision was evaluated. The percentage of RSD was found and calculated within the limits. The results were summarised in the Table 2.

Table 2: Precision method of proposed RP-HPLC method

Drug Mean	Area	%RSD
Ceftalazone	1121169	38
Tazobactum	104558	121

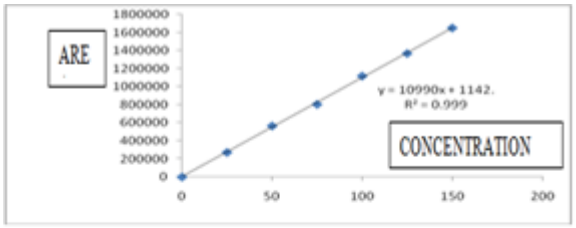
The performance of recovery studies at 50, 100 and 150% levels on the commercial formulations proved the accuracy.% Recoveries of Ceftalazone and Tazobactum ranges from 100.59 to 100.63% were obtained in simultaneous method. The results were shown in the Table 3.

Table 3: Recovery data for Ceftalazone and Tazobactum

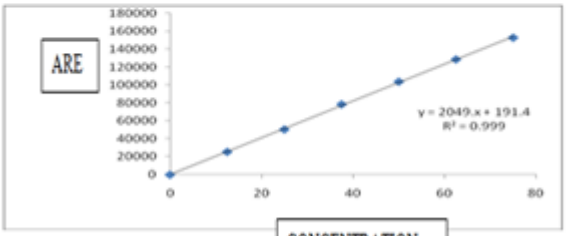
Drug	Spiked Level%	% Recovery	% RSD
Ceftalazone	50	100.69	0.89
	100	100.87	0.69
	150	100.22	0.44
Tazobactum	50	100.77	0.84
	100	100.29	1.30
	150	100.84	0.91

Linearity of Ceftalazone and Tazobactum was established at different concentrations respectively. The calibration curve was plotted in between the concentration and area of both Ceftalazone and Tazobactum (Figure 5a and 5b).

Figure 5: (a and b) Linearity curve of Ceftalazone and Tazobactum



a



b

Six concentrations ranging between 25-150 µg/mL of Ceftalazone and 12.5-75 µg/mL of Tazobactum were chosen for the present study. The correlation coefficient and regression equation for Ceftalazone and Tazobactum was found to be y=10990x+1142.8. And R_2=0.9990 and y=2049.1x+191.44. And R_2=0.9990 respectively. The results were summarized in Table 4.

Table 4: Results of Linearity

S. No	Ceftalazone Conc. (µg/ml)	Peak Area	Tazobactum Conc. (µg/m1)	Peak Area
1	25	272491.7	12.5	25492.67
2	50	565270	25	50439
3	75	804492	37.5	78474.67
4	100	1117693	50	103672.7
5	125	1367615	62.5	128505.3
6	150	1650048	75	152640

Table 5: Robustness Data

Parameters	Changed Condition	Mean Peak Area CEFT	TAZO	USP plate count CEFT	TAZO
Flow rate (ini-Min)	0.9 ml	1167750	116819	2250	6708
	1.0 ml	1164039	110341	2422	7024
	1.1 ml	1061659	106648	2312	6036
Temperature(=5)	25°C	982136	95232	2405	6285
	30°C	1164039	110341	2422	7024
	35°C	1212433	115677	2269	6736
	45:55%v/v	1045012	104193	2391	6054
Mobile phase (± 5%)	50:50%v/v	1164039	110341	2422	7024
	55:45%v/v	1026910	96582	2279	6378

Small deliberate changes in different parameters like flow rate, column temperature and mobile phase composition were not affected by Robustness method. The flow rate effect in mobile phase was studied by changing its volume from 1.0 mL to 0.9 mL and 1.1 mL. The effect of column temperature verified by varying the temperature to ± 5^{o}C. The deliberated variations in all the above parameters cannot show any significant effect on the behavior of chromatographs. The results were summarized in Table 5.

LOD and LOQ of Ceftalazone and Tazobactum were evaluated based on the response of relative standard deviation and calibration curve slope. The detection limits were found to be 0.36 and 0.06 µg/mL for Ceftalazone and Tazobactum respectively. The quantitation limits were found to be 1.09 and 0.19 µg/mL for Ceftalazone and Tazobactum respectively. The results were summarized in the Table 6.

Table 6: Results of LOD and LOQ

Drug	LOD (µg/ml)	LOQ (µg/ml)
Ceftalazone	0.36	1.09
Tazobactum	0.06	0.19

CONCLUSION

A new stability indicating Reverse Phase High Performance Liquid Chromatography method has been designed and developed for estimation of Ceftalazone and Tazobactum in bulk and pharmaceutical formulations. The developed method was validated for the precise, simple, robust and sensitive analysis of Ceftalazone and Tazobactum in both bulk and pharmaceutical formulations. The degradation studies were forcibly conducted as per ICH guidelines. The proposed RP-HPLC method was very sensitive, accurate and simple. This proposed method maintains standards to separate drugs apart from degradation products and excipients found in the pharmaceutical formulations.

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