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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF CEFEPIME AND TAZOBACTAM IN MARKETED FORMULATION

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ABSTRACT

A new precise, accurate, reliable validated method for the determination of Cefepime and Tazobactam has been developed by using reverse phase high performance liquid chromatography (RP-HPLC) in pharmaceutical dosage form. Chromatographic separation was carried out by using mobile phase 0.02M Potassium dihydrogen phosphate: Acetonitrile (95:5v/v, pH-3.0 adjusted with Orthophosphoric acid) on Sunfire C18 (50 x 4.6 mm, 5µ) at a flow rate 0.8ml/min with UV detection at 220nm.The retention times for Cefepime and Tazobactam were 2.243 and 4.910 min respectively and both drugs showed good linearity in the range of 250-750 µg/ml and 31.25-93.75 µg/ml. The proposed method has been successfully applied to pharmaceutical formulation and was validated according to ICH guidelines and method showed good precision with percentage relative standard deviation less than 2%. The percentage recovery for Cefepime and Tazobactam was found between 99.80-101.11% and 100.43-101.14% respectively indicating the proposed method was accurate and precise.

Key words: Cefepime (CEF), Tazobactam (TAZ), RP-HPLC

INTRODUCTION

Cefepime Hydrochloride (CEF) is chemically (6R,7R,Z)-7-(2-(2-aminothiazol-4-yl)-2-(methoxy imino) acetamido)-3-((1-methyl pyrrolidinium-1-yl) methyl)-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylate. It is used in the treatment of urinary tract infections. Cephalosporins disrupt the synthesis of the peptidoglycan layer of bacterial cell wall. [1-2] Tazobactam Sodium (TAZ) is chemically [2S-(2a,3b,5a)]-3-Methyl-7-oxo-3(1H,2,3-triazol-1-ylmethyl)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylicacid 4,4-dioxide sodium salt. It is used in the treatment of bacterial infections. Tazobactam is a compound that inhibits the action of bacterial beta-lactamases. [3-4] Literature survey revealed that few analytical techniques are available for estimation of CEF alone as well as in combine dosage form such as UV ,HPLC, .[5-8] Similarly few analytical methods are available for estimation of TAZ alone and its combination with drugs such as UV and HPLC.[9-12] keeping this objective in mind an attempt has been made to develop and validate the RP-HPLC method for the simultaneous estimation of Cefepime and Tazobactam which would be highly sensitive having good resolution reproducible and cost effective. Various validation aspects of the analysis accuracy, precision, recovery, the limits of detection and quantification etc have been measured as per ICH guidelines. [13]

MATERIALS AND METHOD

Equipment: Chromatographic separation was performed on HPLC system - Water's alliance 2695 with 2996 module Photo Diode Array (PDA) detector equipped with a solvent delivery pump, automatic sample injector and column thermostats. Waters Empower2 software was applied for data collecting and processing.

Chemicals and reagents: Methanol, Acetonitrile (HPLC grade) was used. Buffer used was Potassium dihydrogen ortho phosphate. Reference standards Cefepime and Tazobactam were obtained from Aurobindo Pharma Ltd. Celrim-TZ Injection of CEF (1000mg) and TAZ(125mg) manufactured by Biocon pharmaceuticals Ltd were procured from local market.

Preparation of standard solutions: Accurately weighed 250mg of Cefepime and 31.25mg of Tazobactam each was transferred into a clean and dry 100ml volumetric flask, dissolved with sufficient volume of diluent and sonicated for 5min. The volume made up to 100ml with diluent to obtain 2500µg/ml of Cefepime and 312.5µg/ml of Tazobactam stock solutions. Transfer 2ml of above solution into 10ml volumetric flask and made up to 10ml with diluent.

Preparation of sample solution: 10ml of single dose injection containing 1000mg of Cefepime and 125mg Tazobactam were transferred into a 100 mL volumetric flask, 70mL of diluent was added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.5ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Preparation of buffer: Accurately weighed 2.72gm of Potassium dihydrogen orthophosphate was transferred into a 1000ml of Volumetric flask, about 900ml of milli-Q water was added and sonicated to degas and finally make up the volume with water and 0.6ml of Triethylamine was added. Finally pH is adjusted to 3.0 with dilute orthophosphoric acid solution.

Optimized chromatographic conditions

Flow rate : 0.8ml/min
Column : Sunfire C18, 50 x 4.6 mm, 5µ.
Detector wave length : 220nm
Column temperature : 30°C
Injection volume : 10µL
Run time : 10 min
Diluent : water : Acetonitrile (50:50)

METHOD VALIDATION

System suitability test: This parameter was evaluated before each stage of validation. Six replication injections of standard preparation were injected. Asymmetry, number of theoretical plates and relative standard deviation of peak area were determined.

Linearity: Solutions were prepared containing 250µg/ml, 375µg/ml, 500µg/ml, 625µg/ml, 750µg/ml, concentrations of Cefepime and 31.25µg/ml, 46.87µg/ml, 62.5µg/ml, 78.125µg/ml, 93.75µg/ml, concentrations of Tazobactam which corresponding to 50, 75, 100, 125 and 150% respectively of the test solution concentration. Each solution was injected, linearity was evaluated by linear- regression analysis.

Accuracy: Accuracy was determined by the recovery studies at three different concentrations (corresponding to 50, 100 and 150% of the test solution concentration) by addition of known amounts of standard to pre-analysed sample preparation. For each concentration, three sets were prepared and injected.

Precision: Intraday and interday variations were determined by using six replicate injections of one concentration and analyzed on the same day and different days. Precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements.

Robustness: The robustness was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions. The factors chosen for this study were the flow rate (±0.1ml/min), mobile phase composition (buffer: acetonitrile by 2%).

Limit of detection (LOD) and Limit of quantification (LOQ): LOD and LOQ was calculated from linear curve using formulae

LOD= 3.3 * σ / slope, LOQ= 10 * σ / slope

(Where σ = the standard deviation of the response and S = Slope of calibration curve).

Specificity: Specificity was checked for the interference of impurities in the analysis of blank solution and injecting sample solution under optimized chromatographic conditions to demonstrate separation of both CEF and TAZ from impurities.

RESULTS AND DISCUSSIONS

Several mobile phase compositions were tried to resolve the peak of CEF and TAZ. The mobile phase containing buffer: Acetonitrile in proportion of 95:5v/v was found ideal to resolve the peak of CEF and TAZ. Retention time of CEF and TAZ were 2.243 and 4.910 min respectively (Figure 1&2). Result of assay is shown in Table-1. The proposed method was found to be linear in concentration range 250-750µg/ml for CEF and 31.25-93.75µg/ml for

FEN. The data was shown in Table-2 and Figure-3&4. System suitability parameters were evaluated and results shown in (Table-3), which were within acceptance criteria. The mean percentage recovery for CEF and TAZ was found to be between 99.80-101.11% and 100.43-101.14% respectively, which are well within the limit and hence the method was found to be accurate (Table-4). LOD and LOQ values were 32.26µg/ml and 97.75µg/ml for Cefepime and 3.19µg/ml and 9.67µg/ml for Tazobactam (Table-5). Results of intraday and interday precision were shown in the (Table-6a&6b). The robustness of the method was investigated by varying experimental conditions such as changes in flow rate and mobile phase composition. The result obtained implies method is robust for routine qualitative analysis (Table-7).

CONCLUSION

The proposed RP-HPLC method was validated as per International conference on harmonization (ICH) guidelines, and found to be applicable for routine quality control analysis for the simultaneous estimation of CEF and TAZ using isocratic mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The proposed method is highly sensitive, reproducible, reliable, rapid and specific.

ACKNOWLEDGEMENT

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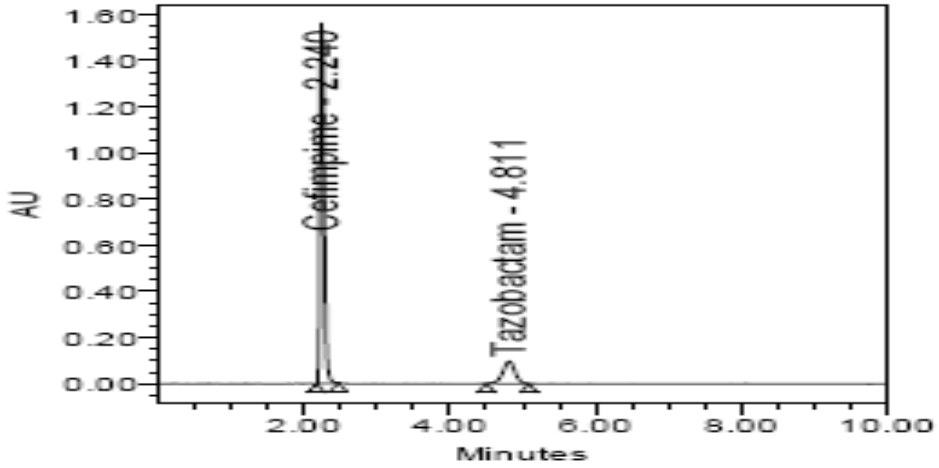

Figure-1: Chromatogram of CEF (500µg/ml) and TAZ (62.5µg/ml) standard

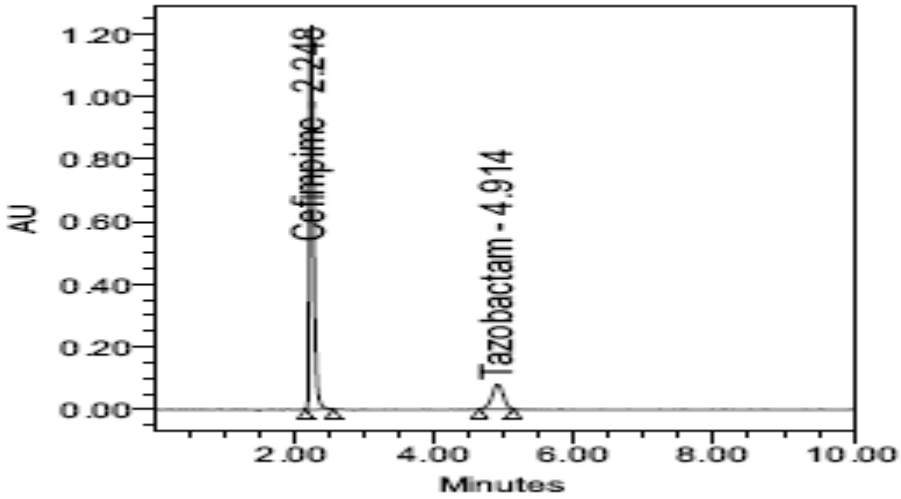

Figure-2: Chromatogram of CEF (500µg/ml) and TAZ (62.5µg/ml) sample

Table -1 Analysis data of formulation (Celrim-TZ)

Injection	Label claim(mg)	Assay (%)
CEF	1000	100.08
TAZ	125	99.74

Table – 2: Result of Linearity

Cefepime		**Tazobactam**	
Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area
0	0	0	0
250	3645928	31.25	621769
375	5394743	46.87	932041
500	7077915	62.5	1289303
625	8672776	78.125	1598881

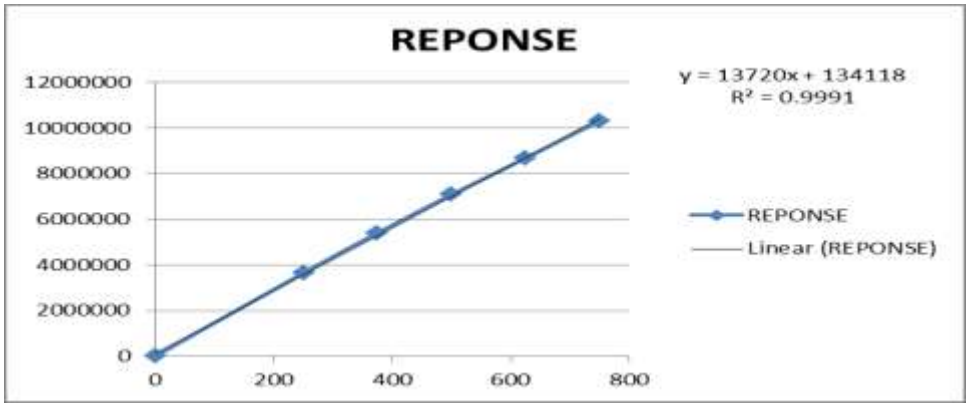

Figure-3: Calibration curve for Cefepime

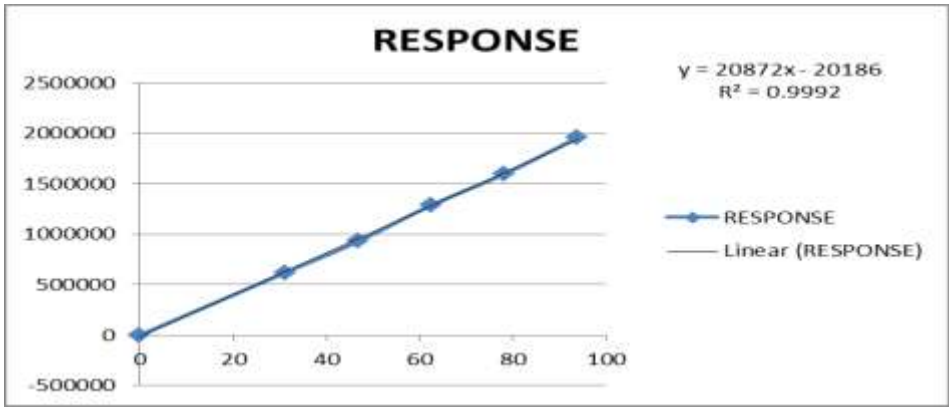

Figure -4: Calibration curve for Tazobactam

Table-3: System suitability studies

Parameters	Cefepime	Tazobactam	Acceptance criteria
Theoretical plates	8635	4785	Not less than 2000
Tailing factor	1.32	0.95	Not more than 2
%RSD	1.3	0.7	Not more than 2

Table-4: Recovery studies for Cefepime and Tazobactam

DRUG	Spiked level%	Amount taken (µg/ml)	Amount found (µg/ml)	Percent recovery n=3	% RSD
CEF	50	500	501.37	100.27	0.23
	100	1000	998.03	99.80	0.24
	150	1500	1516.60	101.11	1.04
TAZ	50	62.5	63.21	101.14	0.65
	100	125	125.60	100.47	1.05
	150	187.5	188.31	100.43	1.53

n- Number of replicate injections

Table-5: LOD and LOQ for Cefepime and Tazobactam

DRUG	LOD (µg/ml)	LOQ (µg/ml)
Cefepime	32.26	97.75
Tazobactam	3.19	9.67

Table-6(a): Results of Intraday Precision

DRUG	Conc. (µg/ml)	Peak area (n=6)	% RSD
CEF	500	6104639	0.95
TAZ	62.5	3091457	1.05

n- Number of replicate injections

Table-6(b): Results of Interday Precision

DRUG	Conc. (µg/ml)	Peak area (n=6)	% RSD
CEF	500	5281381	1.1
TAZ	62.5	854981	1.8

Table-7: Results of Robustness study

S. no	Parameter	Condition	Mean Peak area (n=2)		% RSD	
			CEF	TAZ	CEF	TAZ
1.	Flow rate	0.9 ml/min	5601788	991866	0.37	1.61
		0.7 ml/min	6850951	1165868	0.55	1.24
2.	Mobile phase	97:3 v/v	624436	1079363	0.4	0.63
		93:7 v/v	6081975	1069913	1.2	1.4

RSD – relative standard deviation; n- Number of replicate injections

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