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Validated Chromatographic Method for the Estimation of Ceftazidime and Tazobactam in Pure and Tablet Dosage Form

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ABSTRACT

A simple and rapid chromatographic Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method was developed, optimized and validated for the estimation of Ceftazidime (CEF) and Tazobactam (TAZ) simultaneously in pure and tablet dosage form. The selected drugs were analyzed with the aid of Inertsil C18 (250 × 4.6 mm, 5 µ) column using the combination of 0.1 M disodium hydrogen phosphate buffer: acetonitrile: methanol in the ratio of 40:20:40 v/v/v as mobile phase. The wavelength selected for identification is 229 nm. The range for linearity study was fixed as 120-280 µg/ml for CEF and 15-35 µg/ml for TAZ and the elution times were 2.340 and 4.690 min respectively. The r^2 values for the selected ranges were found to be greater than 0.99. Repeatability studies showed Relative Standard Deviation (%RSD) less than 2 for both the drugs under all selected concentrations. The accuracy values are 98.11-101.84% for CEF and TAZ respectively. Assay values for the marketed formulation were found to be in the range of 98-102%. The Limit of Detection (LOD) and Limit of Quantification (LOQ) values are2.44 µg/ml and 7.39 µg/ml for CEF and 0.66 µg/ml and 2.02 µg/ml for TAZ respectively. The developed method aptly suits for regular analysis of selected drugs.

Keywords: Ceftazidime, Tazobactam, RP-HPLC, Inertsil C18 Column, Validation

INTRODUCTION

Ceftazidime (CEF) (Figure 1) is chemically 1-{[(6R,7R)-7-[(2Z)-2-(2-amino-1,3-thiazol-4-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}pyridin-1-ium [1]. It is mainly used to treat different infections caused by bacteria which include severe and life threatening types. It mainly acts by inhibiting the synthesis of bacterial cell wall due to its affinity for penicillin binding proteins [2].

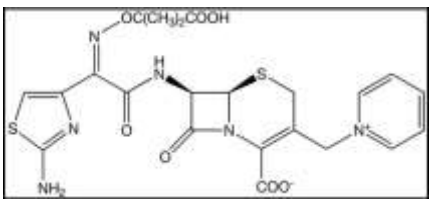

Figure 1: Structure of ceftazidime

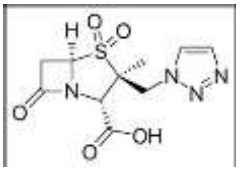

Figure 2: Structure of tazobactam

Tazobactam (TAZ) (Figure 2) is chemically (2S,3S,5R)-3-methyl-4,4,7-trioxo-3-(1*H*-1,2,3-triazol-1-ylmethyl)-4λ-thia-1-azabicycloheptane-2-carboxylic acid [3]. It is a penicillin derivative and has got antibacterial activity as it inhibits β-lactamases of bacteria. It hastens the activity of penicillin's by making them effective against β-lactamases expressing organisms, which would generally deplete penicillin [4].

The selected drugs are available in combination as parenteral formulation (8:1). The formulation is generally prescribed to treat lower respiratory tract infections and dermatological infections. Detailed survey on availability of analytical methods for the combination exposed few analytical methods which include UV [5-9], High Performance Liquid Chromatography (HPLC) [10-15] and High Performance Thin Layer Chromatography (HPTLC) [16,17] methods individually or in combination with other drugs. The present work mainly gives the details of a simple and alternative method for the estimation of CEF and TAZ by Reverse Phase High Performance Liquid Chromatography (RP-HPLC). The developed method was optimized and validated as per the guidelines of International Conference on Harmonisation (ICH) [18].

EXPERIMENTATION

Instrumentation and apparatus

We have employed Waters e 2965 Alliance HPLC system for research work. Inertsil ODS (250 × 4.6 mm, 5 µm) column was used as stationary phase. The chromatograph was equipped with an auto sampler and a Photodiode Array Detector (PDA) detector. Injection system equipped with 20 µl rheodyne port was used for sample administration. The data obtained was analyzed using Empower 2 software.

Chemicals and solvents

Chandra Laboratories, Hyderabad, T.S, India have gifted reference standards of CEF and TAZ. Combitaz injection (Ceftazidime 1000 mg and Tazobactam 125 mg) was purchased from local pharmacy for assay studies. HPLC grade methanol, acetonitrile (E. Merck, Darmstadt, Germany), di sodium orthophosphate AR grade, orthophosphoric acid (Fischer Scientific Chemicals, Mumbai, India.), HPLC water (Milli-QRO water purification system Ltd., Mumbai, India) were used as solvents.

Chromatographic conditions as shown in tabular form:

Chromatograph	Waters HPLC system
Mobile phase	0.1M disodium hydrogen phosphate (pH 3): acetonitrile: methanol (40:20:40% V/V)
Diluent	Mobile phase
Column	Inertsil ODS (250 × 4.6 mm, 5 µm)
Column temperature	40°C
Wave length	229 nm
Injection volume	20 µl
Flow rate	1 ml/min
Run time	6 min
Retention times	2.340 min for CEF; 4.690 min for TAZ

Preparation of phosphate buffer

Accurately weighed and transferred about 3.80 g of disodium orthophosphate and transferred carefully into a one liter standard flask. Sufficient amount of HPLC water was added to dissolve the contents and finally made up to the mark with water. The pH of the solution was adjusted to 3 using dilute Ortho-phthalaldehyde (OPA).

Preparation of mobile phase

To a one liter standard flask 400 ml of above mentioned buffer and 200 ml of acetonitrile was added and mixed well. The resultant solution was made up to the mark with methanol to obtain a mobile phase ratio of 40:20:40 v/v/v. The prepared mobile phase was carefully degassed using a sonicator for a period of 30 min. The solution was finally filtered using 0.45 µ filter under vacuum.

Preparation of CEF and TAZ standard and sample solutions

Standard solution

200 mg of CEF and 25 mg of TAZ reference standards were accurately weighed and transferred into separate 100 ml standard flasks. Sufficient diluents was added to dissolve the contents and finally made up to volume to obtain a concentration of 2000 µg/ml of CEF and 250 µg/ml of TAZ. From these solutions 2.5 ml of solutions were individually transferred in to a 25 ml standard flask to obtain a combination solution of concentration of 200 µg/ml of CEF and 25 µg/ml of TAZ.

Sample solution

Selected formulation is a freeze dried product which is in powdered form equivalent to 1000 mg of CEF and 125 mg of TAZ. From the selected formulation, the powder equivalent to 200 mg of CEF and 25 mg of TAZ was accurately weighed and transferred in to a clean and dry standard flask. Sufficient amount of diluents was added to dissolve the contents and was exposed to sonication for 30 min for efficient dissolution. The resultant solution was filtered and the filtrate was finally made up to the mark with the diluents. 2.5 ml of the mentioned solution was transferred in to a 25 ml standard flask to obtain a concentration of 200 µg/ml of CEF and 25 µg/ml of TAZ.

Injection of solutions in to the chromatograph

20 µl of the standard and sample solutions were injected separately in to the chromatographic system to witness the peaks of CEF and TAZ.

Method validation

The parameters like linearity, range, precision, accuracy, ruggedness, specificity, system suitability, Limit of Detection (LOD) and Limit of Quantification (LOQ) were evaluated to validate the developed method as per the protocols of ICH.

Linearity

Five standard working solutions of CEF and TAZ in the concentration range of 120-280 µg/ml for CEF and 15-35 µg/ml for TAZ respectively, were prepared in triplicate. The prepared samples were administered into the chromatograph under selected/optimized conditions. Calibration plots were constructed by taking concentration on x-axis against mean peak area on the y-axis.

Precision

Method precision: The repeatability for the prepared concentrations was checked using intra-day analysis and inter-day analysis. For this purpose, concentrations at three levels are selected for CEF and TAZ which falls in the linearity range and they were prepared in triplicate and were injected on the same day and on different days. The responses of the chromatograms were interpreted for standard deviations.

System precision: This was used to check the repeatability of the system. This was evaluated by injecting six injections of the target concentration i.e., 200 µg/ml of CEF and 25 µg/ml of TAZ. %RSD values for the repeated injections were calculated and compared with the limits.

Accuracy

Accuracy of the method was tested by adding appropriate amounts of CEF and TAZ marketed sample to the standard sample solution (predetermined/fixed concentration). The data of the fixed concentration results were compared with those of the spiked samples in terms of recovery. To evaluate this parameter, sample solution was spiked over the range of 50%, 100% and 150% with the marketed sample.

Ruggedness

The repeatability of the method was checked by varying the analysts working on the method. This was evaluated by injecting six injections of the target concentration i.e., 200 µg/ml of CEF and 25 µg/ml of TAZ. %RSD values for the repeated injections done by different analysts were calculated and compared with the limits.

Specificity

The excipients/degradants which are supposed to interfere with analytes were studied by administering a placebo sample into the system. The interpretation was made to check whether any interfering peaks are responding at the retention times of the analytes.

Robustness

This validation parameter was evaluated by changing the conditional parameter of the method like pH, flow rate and organic phase concentration. Organic phase ratio was changed in the range of ± 10% i.e., 60 ± 10% and a change of ± 0.1 ml/min variation was made to the flow rate. The output obtained by these changes was interpreted to know the robustness of the method.

System suitability

The system suitability of the method was established. This was tested to prove that the developed method produce results with acceptable accuracy and precision. Different parameters studied includes asymmetric/tailing factor, retention time, number of theoretical plates, resolution etc.

Assay of marketed formulation

The concentration of CEF and TAZ in the selected pharmaceutical formulation was evaluated by comparing the obtained results with those of the standard results. The concentration of the marketed sample was determined by injecting six different injections of the same target concentration.

RESULTS AND DISCUSION

Mobile phase optimization

Different trials were carefully performed to optimize the selected chromatographic conditions for the estimation of CEF and TAZ in combined forms with possible short run time. Mobile phases with different combinations were tried like methanol: buffer, methanol: water, acetonitrile: buffer. Finally, mobile phase with 0.1 M disodium hydrogen phosphate: acetonitrile: methanol (40:20:40% v/v/v, pH 3.0) was fixed, at a flow rate of 1 ml/min. Better symmetry for peaks was achieved under these optimized conditions. Chromatograms of blank and placebo illustrate, no interfering, extra peaks were witnessed at the retention times of CEF and TAZ indicating the specificity of the method (Figure 3). The wavelength was fixed at 229 nm for detection, which showed good response of the detector. The retention time of CEF and TAZ were observed at 2.340 min and 4.690 min respectively (Figure 4).

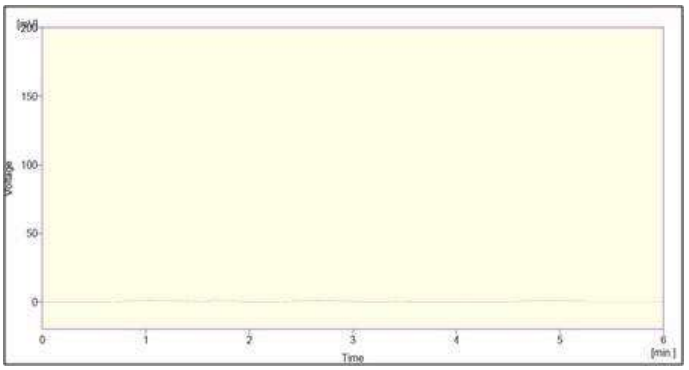

Figure 3: Blank chromatogram for CEF and TAZ

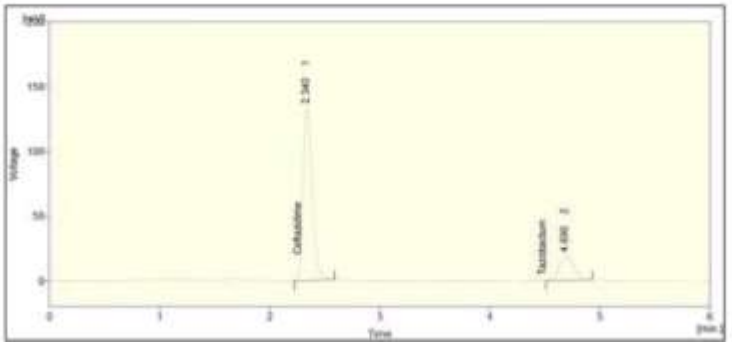

Figure 4: Chromatogram representing well resolved peaks of CEF & TAZ

Validation

Calibration curve was plotted with concentrations on x-axis against respective peak areas. Linearity of the method was observed over the concentration range of 120-280 µg/ml for CEF and 15-35 µg/ml for TAZ; corresponding results were displayed in Table 1, Figures 5 and 6. The repeatability of the method was evaluated and corresponding relative deviations were calculated. %RSD values on the lower side indicate better precision of the method. The results for precision and ruggedness were displayed in Tables 2 and 3. Satisfactory recovery values were obtained in the range of 98.11-100.68% for CEF and 98.88-101.84% for TAZ respectively; results were shown in Table 4. Optimized method was found to be robust when the organic phase percentage was changed to ± 10%; flow rate from 1 ml/min to ± 0.1 ml variation. No drastic changes were observed in peak areas. Even the change in flow rate has slight effect on the retention time of the analyte. The results of the robustness studies were depicted in Table 5. The LOD and LOQ were calculated using standard deviation values of the precision samples and slope obtained from the linearity curve. The LOD for CEF and TAZ were found to be 2.44 µg/ml and 0.66 µg/ml respectively; the LOQ was calculated using the formula thrice the LOD values (or) 3.3 σ/s and were found to be 7.39 µg/ml and 2.02 µg/ml respectively. The values obtained from the system suitability studies for various parameters were depicted in Table 6.

Table 1: Linearity results of ceftazidime and tazobactam

Concentration CEF (µg/ml)	Mean area* ± S.D.	%RSD	Concentration TAZ (µg/ml)	*Mean area ± S.D.	%RSD
120	282599 ± 508.67	0.18	15	16214 ± 186.46	1.15
160	378158 ± 302.53	0.08	20	22746 ± 13.64	0.06
200	483141 ± 5314.55	1.10	25	28271 ± 70.67	0.25
240	583821 ± 291.91	0.05	30	34082 ± 54.53	0.16
280	691882 ± 968.63	0.14	35	40063 ± 124.19	0.31

***Mean of six determinations**

Drug	R^2	Slope	Concentration range (µg/ml)
CEF	0.9991	2465	120-280
TAZ	0.9994	1147	15-35

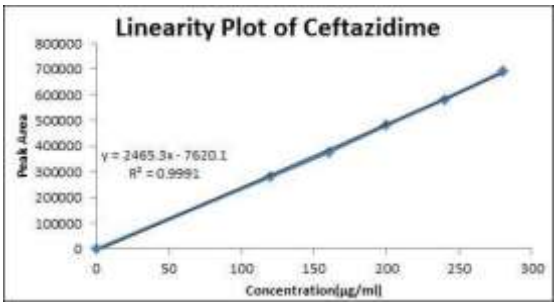

Figure 5: Linearity curve of ceftazidime

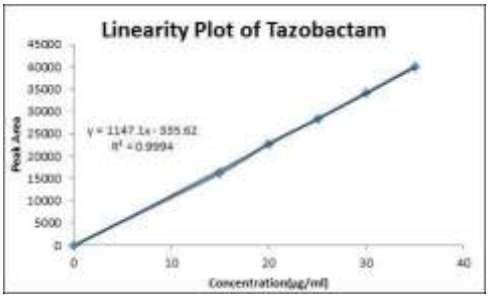

Figure 6: Linearity curve of tazobactam

Table 2: Precision results of ceftazidime and tazobactam

Drug	Concentration (µg/ml)	Intra-day*		Inter-day*	
		Mean area ± S.D.	%RSD	Mean area ± S.D.	%RSD
CEF	120	283189 ± 509.74	0.18	279817 ± 1706.88	0.61
	200	487241 ± 389.79	0.08	489176 ± 4793.92	0.98
	280	701874± 1473.93	0.21	708165 ± 7364.92	1.04
TAZ	15	16098 ± 107.86	0.67	16056 ± 14.45	0.09
	25	28098 ± 14.05	0.05	28901 ± 17.34	0.06
	35	40162 ± 112.45	0.28	40371 ± 327.05	0.81

*****Mean of six determinations

Table 3: Precision results of ceftazidime and tazobactam

Parameter	CEF*	TAZ*
Intra-day precision	0.17-0.38	0.17-0.92
Inter-day precision	1.35-1.80	1.35-1.86
Analyst precision	0.89	0.09
Injection repeatability for RT	0.08	0.17
Injection repeatability for area	0.12	0.21

*****Mean of six determinations

Table 4: Recovery results of ceftazidime and tazobactam

Drug	Concentration (µg/ml)	Amount recovered (µg/ml)	%Recovery	%RSD
CEF	300	300.93	100.31	0.17
	400	402.72	100.68	0.91
	500	490.55	98.11	1.35
TAZ	32.5	32.65	100.49	0.38
	50.0	49.44	98.88	1.18
	62.5	63.65	101.84	0.38

Table 5: Robustness results of ceftazidime and tazobactam

Sample	CEF			TAZ		
	R_t (min)	Area	Tailing factor	R_t (min)	Area	Tailing factor
Standard	2.461	471816	1.269	4.253	29019	1.147
1.1 ml/min	2.218	473631	1.278	4.183	29387	1.132
0.9 ml/min)	2.628	472769	1.289	4.480	30541	1.143
Organic phase (+10%)	2.319	485542	1.269	4.108	28288	1.147
Organic phase (-10%)	2.725	477673	1.283	4.409	29171	1.148

Table 6: Summary of system suitability and validation parameters of ceftazidime and tazobactam

Parameter	Results	
	CEF	TAZ
Retention time (min)	2.340	4.690
Linearity range (µg/ml)	120-280	15-35
Correlation coefficient	0.9991	0.9994
Theoretical plates (N)	3663	5321
Resolution	-	11.37
Tailing factor	1.280	1.171
LOD (µg/ml)	2.441	0.662
LOQ (µg/ml)	7.390	2.021

Assay of marketed formulation

The assay values for the marketed sample were determined and %RSD values were calculated. The results were displayed in Table 7. RSD values on the lower side indicate the suitability of the method for regular and routine quality control analysis. No interfering peaks were observed for excipients/degradants in the assay sample which proves the specificity of the method (Figure 7).

Table 7: Assay results of ceftazidime and tazobactam

Drug	Label claim	Amount found	Mean* %Recovery ± S.D.	%RSD*
CEF	1000 mg	994 mg	99.40 ± 0.09	0.09
TAZ	125 mg	126.34 mg	101.07 ± 0.12	0.11

*Mean of six determinations

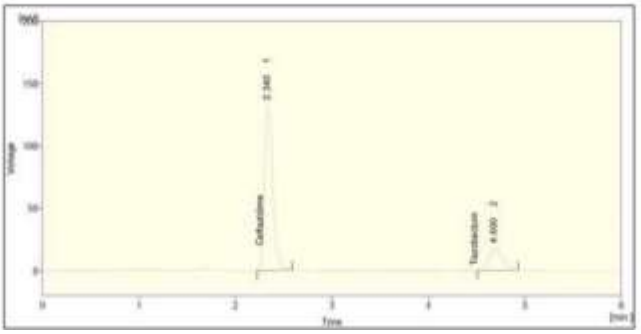

Figure 7: Chromatogram for the sample solution of ceftazidime and tazobactam

CONCLUSION

Developed method was found to be specific, economical, precise, robust, accurate, rapid and robust. Short run time helps in rapid analysis. The RSD values of all the parameters were found to be well within the guidelines limits, indicating the suitability of the method. The assay results found using this method are in agreement with the labeled amounts. Hence, the developed method could be successfully employed for the routine analysis of CEF and TAZ in quality control and laboratory purposes.

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