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Article

Quantification and stress degradation studies of cefepime/ tazobactam in dry injection form by an RP-HPLC method

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A simple, specific, precise, accurate, linear, rapid, economic and validated stability indicating an RP-HPLC method for the simultaneous quantification of cefepime and tazobactam in a dry injection dosage form has been developed. Separation was performed on a 5 µm ACE C_{18} column with phosphate buffer, pH adjusted to 4.5 with phosphoric acid: methanol (70:30) at a flow rate of 1 mL/min and at a temperature of 25 °C. Regression analysis showed linearity at a detector wavelength of 290 nm in the range of 200-600 µg/mL for cefepime and 25-75 µg/mL for tazobactam. All of the analytes were adequately resolved with acceptable tailing. The percentage content found for cefepime was 99.98% and of tazobactam was 99.49% in the parenteral formulation. The method was validated in terms of linearity, precision, accuracy, specificity, robustness and system suitability according to ICH guidelines. Stress degradation studies were performed on the placebo and drug products, drugs of interest were well resolved from the degradation products. The developed method was effectively applied for the simultaneous quantification of cefepime and tazobactam in a dry injection formulation.

Uniterms: High performance liquid chromatography/quantitative analysis. Cefepime/quantificação. Tazobactam/quantificação. Dry injection formulation/quantitative analysis. Pharmaceutical formulations/ degradation studies.

Desenvolveu-se método específico, preciso, exato, linear, rápido e econômico, de validação de estabilidade, indicando o método de CLAE-FR para a quantificação simultânea de cefepima e tazobactam na forma de dosagem injetável seca. A separação foi realizada em coluna C18 de ACE 5 mM com tampão fosfato, pH ajustado para 4,5 com ácido fosfórico:metanol (70:30), em fluxo de 1 mL/min e temperatura de 25 °C. A análise de regressão mostrou linearidade no detector de comprimento de onda de 290 nm, na faixa de 200-600 µg/mL, para cefepima, e 25-75 µg/mL, para tazobactam. Todos os analitos foram, adequadamente, resolvidos com cauda aceitável. O teor percentual encontrado na formulação parenteral foi de 99,98%, para cefepima, e de 99,49%, para o tazobactam. O método foi validado em termos de linearidade, precisão, exatidão, especificidade, robustez e adequação do sistema de acordo com as diretrizes ICH. Estudos de degradação por estresse foram realizados no grupo placebo e nos medicamentos e os fármacos de interesse foram bem resolvidos a partir dos produtos de degradação. O método desenvolvido foi efetivamente aplicado para quantificação simultânea de cefepima e tazobactam na formulação injetável seca.

Uniterms: CLAE-FR. Cromatografia líquida de alta eficiência/fase reversa/análise quantitativa. Cefepima/ quantificação. Tazobactam/quantificação. Formulação injetável seca/análise quantitativa. Formulações farmacêuticas/estudo de degradação.

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INTRODUCTION

ICH and WHO recommend that analysis of pharmaceutical finished products during stability testing should be conducted using a validated stability-indicating method. In this study, ICH and WHO recommendations were therefore kept in mind for the simultaneous estimation of cefepime (CEF) and tazobactam (TAZ).

CEF is a fourth-generation, semi-synthetic, broad spectrum, cephalosporin. Chemically, it is 1-[[(6*R*,7*R*)-7-[2-(2-amino-4-thiazolyl)-glyoxylamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride, 72-(Z)-(*O*-methyloxime), monohydrochloride, monohydrate (Figure 1). It is used in the treatment of moderate-to-severe infections, such as pneumonia, uncomplicated urinary tract infections, skin and soft tissue infections, intra-abdominal infections and febrile neutropenia.

TAZ is semi-synthetic parenteral penicillin. It is a β-lactamase inhibitor with a broad spectrum of antibacterial activity against most gram positive, gram negative aerobic and anaerobic bacteria (Ghafur, Ashwini, Priyadarshini, 2012.). Chemically, it is known as (2*S*,3*S*,5*R*)-3-methyl-7-oxo-3-(1*H*-1,2,3-triazol-1-ylmethyl)-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid 4, 4-dioxide (Figure 2).

An extensive literature search was carried out on analytical methods that were developed to estimate the combination of CEF and TAZ. The literature survey revealed that several spectrophotometric methods had been reported for the determination of CEF alone as well as for stability and degradation studies (Singh, 2013; Moreno, Salgado, 2012; Chahana, Harsha, Chhaganbhai, 2013). CEF in combination with other drugs can be estimated using numerous liquid chromatography methods (Palacios *et al*., 2005; Nanda *et al*., 2012; Pedroso, Salgado, 2014). TAZ was also successfully determined by HPLC methods.

However, there is no RP-HPLC method available for stability indicating the validated simultaneous quantification of CEF and TAZ in combination so far. Hence, this research work was carried out with the objective of developing a simple RP-HPLC method for the simultaneous quantification of CEF and TAZ in bulk as well as in a dry injection formulation and its application to stress degradation studies.

MATERIAL AND METHODS

Instruments

The HPLC system consisted of a Waters E2695 with EMPOWER software, a POLMAN pH meter, model LP139SA, and a Sartorius analytical Balance, model BSA224S-CW.

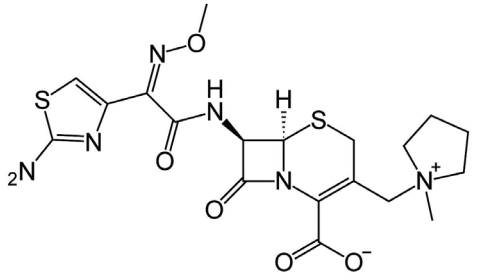

FIGURE 1- Chemical structure of CEF.

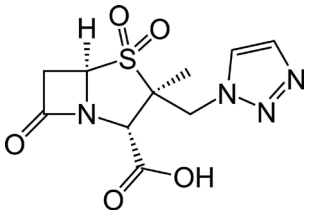

FIGURE 2 - Chemical structure of TAZ.

Chemical reagents

Methanol was of HPLC grade, while all other chemicals and reagents, including orthophosphoric acid, potassium dihydrogen phosphate, and hydrochloric acid, were of analytical reagent grade and were supplied by Merck. Double-distilled water and milli-Q water was used for all of the experiments, as appropriate. Filtration of the mobile phase was performed using 0.45 mm nylon filters (Millipore, USA). CEF and TAZ drug samples were gifted by Aurobindo Pharma Pvt. Ltd. (Hyderabad, India).

Preparation of standard solutions

Standard stock solutions of the two drugs were prepared by accurately weighing 40 mg CEF and 5 mg TAZ and then dissolving this in a few mL of methanol. The volume of this solution was then made up to 100 mL with the mobile phase. The stock solution was diluted with mobile phase to obtain final concentrations equal to 400 µg/mL CEF and 5 µg/mL TAZ. The solution was filtered using a nylon filter before analysis.

Preparation of sample solution

The dry injection formulation containing 1000 mg of CEF and 125 mg of TAZ was reconstituted with

sterile water to a volume of 10 mL. From this, 2 mL was transferred into a 100 mL volumetric flask and diluted up to the mark with mobile phase to obtain a concentration equal to 2000 µg/mL of CEF and 250 µg/mL of TAZ. The above solution was further diluted with mobile phase to obtain final concentrations within the linearity range to quantify them using the proposed RP-HPLC method.

Method validation

The proposed method was validated as per ICH guidelines for the following parameters: linearity and range, precision, accuracy, specificity, ruggedness, robustness, limit of detection (LOD), limit of quantitation (LOQ), system suitability and forced degradation studies.

Linearity

Appropriate aliquots of standard stock solution were diluted with diluent to obtain final concentrations of CEF and TAZ in the range of 200-600 µg/mL and 25-75 µg/mL, respectively. A 10 µL aliquot of each sample was injected six times for each concentration level and a calibration curve was constructed by plotting the average peak area versus the drug concentration.

Precision

Precision was checked for both the system and the developed method. The system precision was checked using standard CEF and TAZ solutions. The retention time and area of ten determinations were measured and the RSD (Relative Standard Deviation) was calculated. A homogenous sample of a single batch was analysed six times to determine the precision of the developed method. Percentage assay values and the RSD of the assay were calculated.

Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy of the proposed method was ascertained on the basis of a recovery study performed using the standard addition method. Accuracy was performed at three different levels for CEF and TAZ. A known quantity of CEF and TAZ standard was spiked at 50%, 100% and 150% levels into the placebo. Analyses of samples were performed in triplicate for each level. The percentage recovery was calculated from the obtained results.

Specificity

The analytes should show no interference from other extraneous components and should be well resolved from them. Specificity is the procedure used to detect the analytes quantitatively in the presence of a component that may be expected to be present in the sample matrix.

Robustness

As defined by the ICH, the robustness of an analytical procedure refers to its capability to remain unaffected by small and deliberate variations in method parameters. The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. Different parameters that were altered to evaluate robustness of the method included flow rate by ±0.2 mL/min, temperature by ± 5 °C and mobile phase composition.

Forced degradation studies

To determine whether the analytical method was able to indicate stability, a CEF and TAZ formulation was stressed under various conditions to conduct forced degradation studies. Intentional degradation was attempted in stress conditions of acidic (0.1 N HCl), basic (0.1 N NaOH), neutral (water), oxidative degradation (1% H_2O_2) and thermal treatment (heated at 80 °C to evaluate the ability of the proposed method to separate CEF and TAZ from its degradation products). The test preparation was subjected to acid stress degradation by treating 5 mL of sample with 5 mL of 0.1 N HCL. The contents were mixed well and constantly stirred for 30 min and then neutralised with 5 mL of 0.1 N NaOH. Then, it was diluted as per the test procedure and injected into the HPLC system. Similarly, alkali (NaOH), peroxide degradation (H_2O_2), neutral degradation (60 °C for 60 min) and thermal degradation (80 °C for 30 min) tests were carried out.

RESULTS AND DISCUSSION

For the method development, several trials were carried out and the final optimised chromatographic conditions for the separation and quantification of CEF and TAZ in the bulk and dry injection formulation were reported. Preliminary studies involved using an ACE C_{18} column, with a 5 µm size of the packing material, and several mobile phase compositions for the effective separation of these two drugs. Using the ACE C_{18} Column, 5 µm size, eluted with phosphate buffer of pH 4.5:MeOH

(70:30) by isocratic elution at a flow rate of 1 mL/min and a detection wavelength of 290nm with injection volume of 10 µL at 25 °C afforded the best separation of these analytes. The chromatogram of the optimised method is shown in Figure 3. The system suitability parameters are shown in Table I.

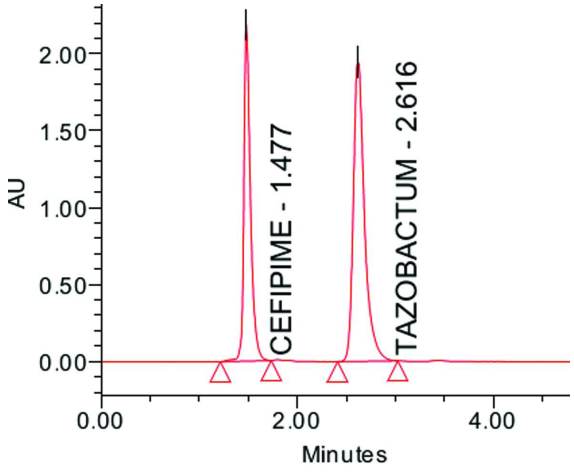

FIGURE 3 - Chromatogram obtained after optimisation.

TABLE I - Results of system suitability parameters

Sample	Retention time	Area	USP resolution	USP Tailing	Plate count
CEF	1.477	6286610		1.480	3671
TAZ	2.616	4169897	7.225	1.442	4969

From the results of the assay study, the content of CEF was found to be 999 mg/mL (label claim percentage was 99.98%) while TAZ was 124.5 mg/mL (label claim percentage was 99.49%) (Table II).

Linearity

Linear calibration plots for the proposed method were obtained in concentration ranges of 200-600 µg/mL for CEF and 25-75 µg/mL TAZ. The linear regression equation for CEF was y=15625x-5844, with a correlation coefficient of 0.999 (Figure 4), and for TAZ it was y=82449x-9735, with a correlation coefficient of 0.999 (Figure 5).

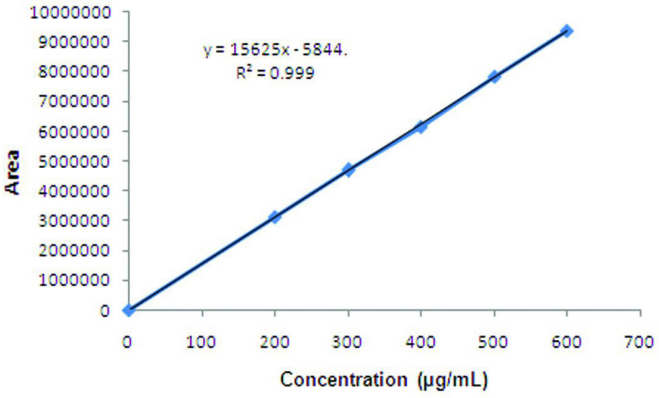

FIGURE 4 - Linearity plot of CEF.

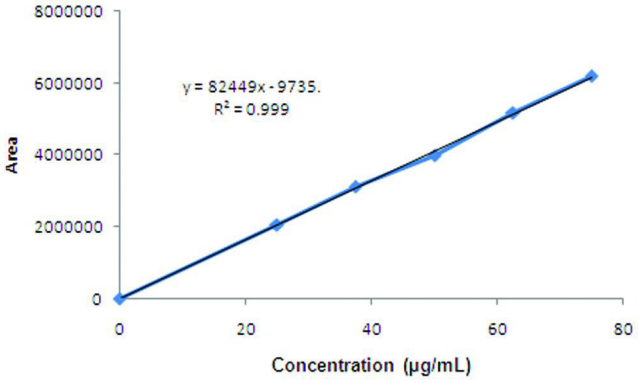

FIGURE 5 - Linearity plot of TAZ.

Accuracy

The accuracy of the method was determined by the standard addition method. Three levels of solutions (50, 100 and 150%) of the nominal analytical concentrations were prepared. Percentage recoveries along with standard deviations and relative standard deviations for each analyte (n=6) are given in Table III. Recovery studies showed the method to be highly accurate and suitable for the intended use.

TABLE II - Assay of CEF and TAZ in formulation

Formulation	CEF				TAZ			
	Label claim (mg)	Amount found (mg) A.M± SD	%Assay	% RSD	Label claim (mg)	Amount found(mg) A.M± SD	%Assay	%RSD
XEPIME TAZ	1000	999.66±0.152	99.98	0.0152	125	124.5±0.458	99.49	0.367

TABLE III - Accuracy of the proposed HPLC method

Amount of test solution	Recovery level (%)	Recovery of analyte	Amount of std added	Theoretical content (µg/mL)	Amount found(µg/mL) A.M± SD (n=3)	Recovery (%)	% RSD
400 µg/mL CEF 50 µg/mL TAZ	0	CEF	0	400	398.47±1.917	99.61	0.481
		TAZ	0	50	49.59 ±0.002	99.18	0.041
	50	CEF	200	600	599.95±0.630	99.91	0.105
		TAZ	25	75	74.67±0.110	99.99	0.147
	100	CEF	400	800	798.64±0.076	99.92	0.001
		TAZ	50	100	99.63±0.574	99.63	0.576
	150	CEF	600	1000	998.43 ±0.165	99.48	0.016
		TAZ	75	125	124.46±0.450	99.57	0.362

Precision

The system and the developed method produced satisfactory precision results. The replicate estimation of a dry injection formulation analysed by the proposed method yielded quite consistent results, indicating the repeatability of the method. The study showed %R.S.D.<2 for both CEF and TAZ (Table IV).

TABLE IV - Results of method precision of CEF and TAZ

Injection no.	CEF		TAZ	
	Retention Time (min)	Peak Area	Retention Time (min)	Peak Area
1	1.480	6264001	2.638	4139862
2	1.480	6266419	2.637	4132529
3	1.481	6269715	2.638	4131457
4	1.479	6262821	2.637	4137014
5	1.481	6267552	2.640	4136180
6	1.480	6266298	2.640	4133972
Mean		6266134		4135169
SD		2468.5		3118.70
% RSD		0.039		0.075

Limit of detection and limit of quantification

The Limit of detection (LOD) and Limit of Quantification (LOQ) were determined by making different solutions with decreasing concentrations of analytes. LOD was found to be 0.475 µg/mL and 0.148 µg/mL for CEF and TAZ, respectively (S/N ratio 3:1). LOQ was found to be 1.585 µg/mL and 0.494 µg/mL for CEF and TAZ, respectively (S/N ratio 10:1).

Robustness

Robustness of the method was performed by slightly varying the chromatographic conditions. The results showed a negligible effect on the chromatographic parameters by slight variations in chromatographic conditions with respect to mobile phase, temperature and flow rate. Results are presented in Table V.

Stress degradation studies

All of the stress conditions applied were capable of degrading both of the drugs. The degradation data are shown in Table VI; it was shown that CEF degradation was more efficient in acidic than other stress conditions and TAZ degradation was lower in all of the stress conditions. The drugs of interest peaks were well separated from the degradation product peaks and the resolution was found to be more than 2. Hence, the developed RP-HPLC method has the ability to quantify CEF and TAZ in the presence of degradation products.

CONCLUSION

There is currently no RP-HPLC method that is capable of the simultaneous estimation of CEF and TAZ in the injection dosage form. Also, no stress degradation studies have been performed for this combination. The developed method was simple, rapid, precise, accurate and economical, and can be employed for the routine estimation of CEF and TAZ in injection dosage. All of the validation parameters were found to be highly acceptable indicators for specificity, linearity and range, accuracy, precision, ruggedness, LOD, LOQ and robustness. The stress degradation studies of CEF and TAZ were checked by the proposed method and the degradation peaks were

TABLE V - Robustness study results of CEF and TAZ

Drug	Parameter	Retention time	Area	USP Tailing	USP Plate Count
	Change in mobile phase				
CEF	10% decrease	1.454	10091661	1.519	3352
	10% increase	1.449	10055239	1.594	4470
TAZ	10% decrease	2.392	15275720	1.697	3926
	10% increase	2.671	15297484	1.554	4856
	Change in flow rate (±2 mL/min)				
CEF	0.8	1.836	12533544	1.451	3207
	1.2	1.231	83970181	1.557	3156
TAZ	0.8	3.262	19055459	1.497	4575
	1.2	2.200	12759170	1.575	4371
	Change in Temperature (±5 °C)				
CEF	30	1.474	10059193	1.451	3207
	30	1.467	10147134	1.497	3156
TAZ	40	2.631	15300002	1.557	4575
	40	2.580	15271258	1.575	4371

TABLE VI - Results of forced degradation studies

Nature of stress	%Assay CEF	%Assay TAZ	%Net degradation CEF	%Net degradation TAZ
Neutral	98	92	1.98	4.83
Acid	73	97	27.40	2.18
Base	96	94	3.59	4.72
Peroxide	83	96	16.56	3.21
Thermal	87	97	12.65	2.17

well separated from the sample peaks. The developed method was successfully applied for the simultaneous quantification of CEF and TAZ in a dry injection formulation.

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