

Simultaneous Spectrophotometric Estimation of Cefepime and Tazobactam in Pharmaceutical Dosage Form

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Abstract: Two simple, accurate and reproducible spectrophotometric methods have been developed for the simultaneous estimation of Cefepime and Tazobactam in pharmaceutical dosage forms. The first method involves determination using the Vierodt's Method (Simultaneous Equation Method); the sampling wavelengths selected are 232 nm and 262 nm over the concentration ranges of 5-50 µg/mL and 2.5-17.5 µg/mL for Cefepime and Tazobactam respectively. The second method involves determination using the Multicomponent Mode Method; the sampling wavelengths selected are 232 nm and 262 nm over the concentration ranges of 5-50 µg/mL and 2.5-17.5 µg/mL for Cefepime and Tazobactam respectively. The results of the analysis were validated statistically and recovery studies were carried out as per ICH guidelines.

Key Words: Cefepime, Tazobactam, Vierodt's Method (Simultaneous Equation Method) and Multicomponent Mode Method.

Introduction

Cefepime (CEF) 1-[[[(6R,7R)-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), mono-hydrochloride, monohydrate salt is official in IP, BP and USP^[1,2,3]. Literature survey reveals several spectroscopic^[4], HPLC^[5] and HPTLC methods for the estimation of CEF individually as well as in combination with other drugs^[6].

Tazobactam (TZB) is chemically 4-Thia-1-azabicyclo [3, 2, 0] heptane 2-carboxylate- (2S,3S,5R)-3-methyl-7-oxo-3-(1H-1,2,3-triazol-1-ylmethyl)-4, 4 dioxide sodium salt. Literature survey reveals UV spectroscopic^[7] and HPLC^[8] methods for the estimation of TZB individually as well as in

combination with other drugs^[9,10]. CEF and TZB are available in combined pharmaceutical dosage form for the treatment of lower respiratory tract infections, skin infections, and urinary tract infections and usually in pediatric infections^[11]. Not a single UV or HPLC method is reported so far for the simultaneous analysis of CEF and TZB in their combined dosage form. So a need was felt to develop new methods to analyze the drugs simultaneously. A successful attempt has been made to estimate the two drugs simultaneously by UV spectrophotometric analysis. This paper describes two simple, rapid, accurate, reproducible and economical methods for the simultaneous determination of CEF and TZB in parenteral formulations using Vierodt's Method (Simultaneous Equation Method) and Multicomponent Mode Method.

Experimental

Instrumentation:

A Shimadzu UV/Visible spectrophotometer, model 1700 (Japan) was employed with spectral bandwidth of 2 nm and wavelength accuracy of ± 0.5 nm, with automatic wavelength correction was employed. A Shimadzu electronic analytical balance (AX-200) was used for weighing the sample. An ultrasonic cleaner (Art No.400014CL) was used for sonicating the injection sample solution.

Reagents and Chemicals:

Analytical pure samples of CEF (Hindustan Antibiotic Limited, Pimpri, Pune, India) and TZB (Gensen Laboratories, Mumbai) were used in the study. The pharmaceutical dosage form used in this study was Magnova (Lyka Labs Limited, Ankaleshwar; Marketed by LUPIN LTD. Mumbai, India) labeled to contain 1000 mg CEF and 125 mg of TZB.

Preparation of Standard Stock Solution:

Standard stock solutions (100 μ g/mL) of CEF and TZB were prepared by dissolving separately 10 mg of drug each in 100 ml 0.1M NaOH. The working standard solutions of these drugs were obtained by dilution of the respective stock solution with 0.1M NaOH.

Preparation of Sample Stock Solutions:

An accurately weighed powder sample equivalent to 40 mg of CEF was transferred to a 100 ml volumetric flask and dissolved in 0.1M NaOH and sonicated for 15 minutes and volume made to 100ml with 0.1M NaOH. It was then filtered through Whatmann filter paper No.41. The solution was suitably diluted with 0.1M NaOH to obtain sample solutions containing CEF and TZB in the concentrations ratio of 8:1 μ g/mL respectively as in the formulation. The final concentrations are 40 μ g/mL of CEF and 5 μ g/mL of TZB.

Method A:

Vierodt's Method (Simultaneous Equation Method)

Construction of calibration curve

For the Vierodt's Method (Simultaneous Equation Method), 232nm, and 262nm were selected as the two sampling wavelengths. Fig.1 represents the overlain UV spectra of CEF and TZB. CEF and TZB exhibited linearity with absorbances in the range of 5-50 μ g/mL and 2.5-17.5 μ g/mL at their respective selected wavelengths. Co-efficient of correlation was found to be 0.9988 and 0.9978 for CEF and TZB respectively. The optical characteristics and regression values for the calibration curves are presented in Table 1. For

simultaneous estimation of CEF and TZB, mixed standards containing CEF and TZB in a concentration ratio of 8:1 μ g/mL each were prepared by appropriate dilution of the standard stock solutions with 0.1M NaOH. The absorbances of the mixed standard solutions were measured at the selected wavelengths. A set of two simultaneous equations were used for obtaining the concentrations of CEF and TZB are as follows;

$$C_x = \frac{A_2 a_{y_1} - A_1 a_{y_2}}{a_{x_2} a_{y_1} - a_{x_1} a_{y_2}} \dots \text{Eq. (i)}$$

$$C_y = \frac{A_1 a_{x_2} - A_2 a_{x_1}}{a_{x_2} a_{y_1} - a_{x_1} a_{y_2}} \dots \text{Eq. (ii)}$$

Where, A_1 and A_2 are absorbances of mixture at 232.0 nm and 262.0 nm respectively, a_{x_1} and a_{x_2} are absorptivities of CEF at λ_1 and λ_2 respectively and a_{y_1} and a_{y_2} are absorptivities of Tazobactam at λ_1 and λ_2 respectively. C_x and C_y are concentrations of CEF and Tazobactam respectively. The concentration of CEF and TZB in mixed standard and injection formulation can be obtained by solving equation (i) and (ii).

Method B:

Multicomponent Mode Method

For the analysis of CEF and TZB by multicomponent method of analysis, the multicomponent mode of the UV visible spectrophotometer was used. For multicomponent method of analysis, 232nm, and 262 nm were selected as the two sampling wavelengths for CEF and TZB respectively. The drugs showed linearity in the concentration ranges of 5-50 μ g/mL, 2.5-17.5 μ g/mL with regression coefficient (r^2) values of 0.9991, 0.9963 for CEF and TZB respectively. Six mixed standards in ratio of 8:1 μ g/mL showing linearity within the Beer's concentration range of CEF and TZB were prepared by appropriate dilution of standard stock solutions (100 μ g/mL). In multicomponent mode of the instrument, the mixed standards were scanned over the range of 190-400 nm at the selected sampling wavelengths. The overlain spectra of the six mixed standards were then employed to determine the concentration of the drugs in sample solutions by analysis of the spectral data of sample solution with reference to that of mixed standards.

Assay of Injection Formulation:

Powder equivalent to 40 mg of CEF and 5 mg of TZB was weighed and dissolved in 100 mL 0.1M NaOH with the aid of ultrasonication for 15 min. The solution was then filtered through Whatmann filter paper No.41 and diluted further to obtain final concentration of 40 µg/mL of CEF and 5 µg/mL of TZB. The sample solutions were analyzed as per the procedure for mixed standards. The concentrations of each drug in sample solutions were calculated using equations (I) and (II) for the Vierodt's Method (Simultaneous Equation

Method) and using the multicomponent mode of the instrument for the Multicomponent method of analysis. The proposed methods were validated as per ICH guidelines [12]. The accuracy of the proposed methods was determined by performing recovery studies at 80%, 100% and 120% of the test concentration. The results of the analysis and statistical validation data of the injection formulation are given in table 1. The statistical validation data of recovery study are given in table 2.

Table 1: Optical Characteristics and Validation Data of CEF and TZB.

Parameters	CEF		TZB	
	Method-A	Method-B	Method-A	Method-B
Working wavelengths	232nm	232nm	262nm	262nm
Beer-Lamberts Law range (µg/mL)	5-30	5-30	2.5-17.5	2.5-17.5
Precision*				
Interday (%RSD)	0.15013	0.24562	0.1760	0.20046
Intraday (%RSD)	0.05645	0.1623	0.2322	0.21193
LOD (µg/mL)*	0.03054	0.20058	0.2308	0.07453
LOQ (µg/mL)*	0.09255	0.60782	0.06994	0.22587
Regression Values:				
I. Slope*	0.0411	0.05416	0.0530	0.08766
II. Correlation Coefficient (r ²)*	0.9988	0.9991	0.9978	0.9963

*Denotes average of five estimations.

Method A – Vierodt's Method (Simultaneous Equation Method)

Method B – Multicomponent Mode Method.

Table 2: Statistical Validation Data of Injection Formulation

Results of Commercial Sample Analysis

Component	Method	Labeled Drug (Mg/vial)	Amount obtained (mg)	% Amount Found	S.D.*	% R.S.D.*
CEF	A	1000	997.3	99.73	0.02152	0.4302
	B	1000	999.1	99.91	0.01883	0.3786
TZB	A	125	125.4	100.32	0.01696	0.3394
	B	125	125.7	100.56	0.01730	0.3462

The % drug obtained and % recovery value are mean of five determinations.

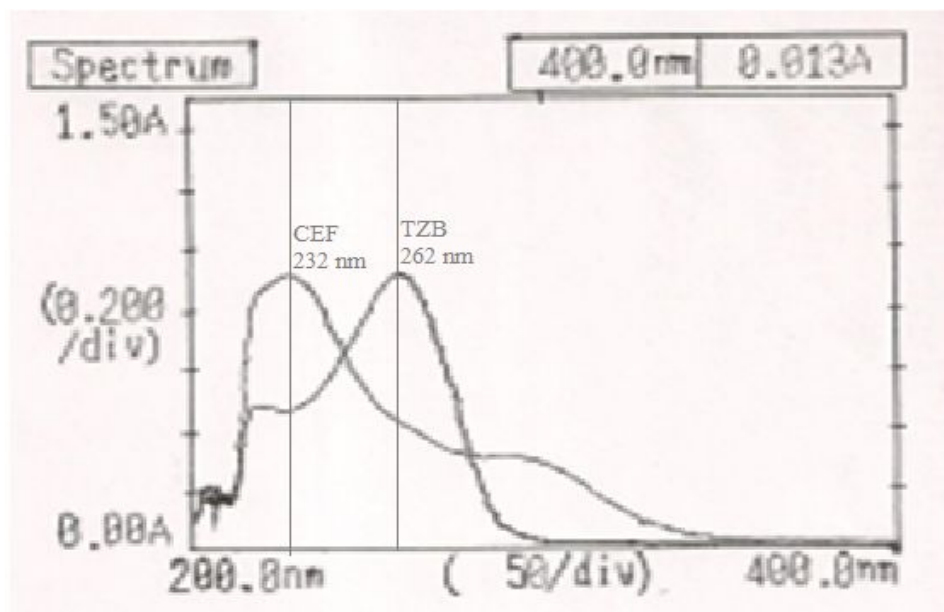
S.D.* = Standard deviation, n= 5, RSD= Relative standard deviation.

Injection Formulation, Magnova, manufactured by Lyka Labs Limited, Ankaleshwar; Marketed by LUPIN LTD. Mumbai, India.

Table 3: Statistical Validation of Recovery Studies

Level of % Recovery	Methods	% Recovery*		% R.S.D.*	
		CEF	TZB	CEF	TZB
80	A	99.82	100.43	0.3427	0.330
	B	99.78	100.02	0.4019	0.1379
100	A	100.09	100.51	0.2901	0.4722
	B	99.73	100.46	0.4738	0.3461
120	A	99.69	100.37	0.0910	0.3386
	B	100.11	99.96	0.3409	0.1792

*Denotes average of three estimations at each level of recovery.

Fig.1: Overlain Spectra of CEF and TZB in Vierodt's Method (Simultaneous Equation Method).

Results and Discussion:

Under the experimental conditions described, calibration curve, assay of injection and recovery studies were performed. The developed methods were validated as per ICH guidelines for linearity, repeatability, intermediate precision (inter-day and intra-day precision studies), LOD, LOQ as shown in Table 1. The mean % content of 99.73% and 99.91% formulation by the developed methods were 100.32% and 100.56% respectively (Table 2). The mean % recoveries of CEF and TZB were found to be 99.87% and 100.29 % respectively (Table 3). The ruggedness of the developed methods was determined by evaluating the effect of change in instruments and analysts on the % mean content of drugs.

Conclusion:

CEF and TZB are available in combined pharmaceutical dosage form for the treatment of Lower respiratory tract infections, urinary tract infections, skin infections, etc. Here, two simple UV

spectrophotometric methods (Vierodt's Method (Simultaneous Equation Method), Multicomponent Mode Method) were developed for their simultaneous analysis. The standard deviation, RSD and standard error calculated for the methods are low, indicating high degree of precision of the methods. The RSD is also less than 2% as required by ICH guidelines. The % recovery was between 98- 102% indicating high degree of accuracy of the proposed methods. The developed methods are simple, rapid, precise, accurate and can be employed for the routine estimation of CEF and TZB in both bulk and injection dosage form.

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