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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF CEFEPIME AND TAZOBACTAM IN COMBINED DOSAGE FORM BY AREA UNDER CURVE AND Q-ANALYSIS

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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 AUC Method (Area Under Curve Method); the sampling wavelengths selected are 235-225 nm and 265-255 nm over the concentration ranges of 5-50µg/mL and 2.5-15 µg/mL for Cefepime and Tazobactam respectively. The second method involves determination using the Q-Analysis Method (Absorbance Ratio Method); the sampling wavelengths selected are 230 nm and 247 nm over the concentration ranges of 5-50 µg/mL and 2.5-15 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.

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), monohydrochloride, 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 (TAZ) 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 TAZ individually as well as in combination with other drugs [9,10].
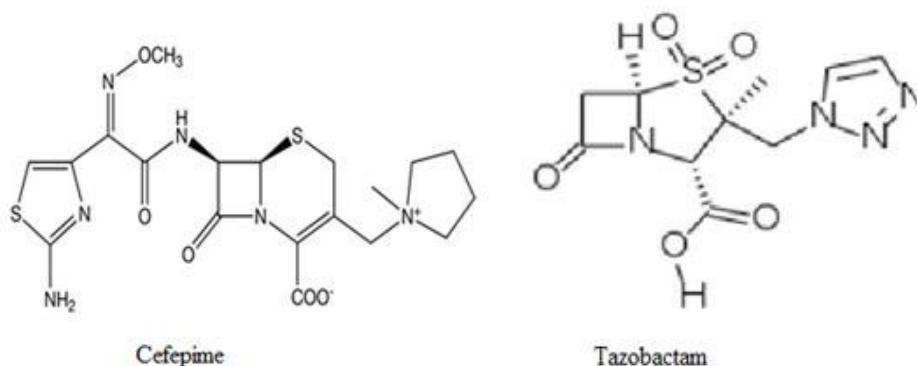
Cefepime   Tazobactam

CEF and TAZ 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 TAZ 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 TAZ in parenteral formulations using AUC Method (Area Under Curve Method) and Q-Analysis Method (Absorbance Ratio Method).

EXPERIMENTAL

Instrumentation:

Shimadzu UV/Visible spectrophotometers, model 1601 and 1700 (Japan) with spectral bandwidth of 2 nm and wavelength accuracy of ± 0.5 nm, with automatic wavelength correction were 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 TAZ (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 Limited, Mumbai, India) labeled to contain 1000 mg CEF and 125 mg of TAZ.

Preparation of Standard Stock Solution:

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

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 KOH and sonicated for 10 minutes and volume made to 100ml with 0.1M KOH. It was then filtered through Whatmann filter paper No.41. The solution was suitably diluted with 0.1M KOH to obtain sample solutions containing CEF and TAZ in the concentrations ratio of 8:1 respectively as in the formulation. The final concentrations are 40 µg/mL of CEF and 5 µg/mL of TAZ.

Method A:

AUC Method (Area Under Curve Method)

Construction of calibration curve

For the AUC Method, 235-225nm, and 265-255nm were selected as the two sampling wavelengths.

Fig.1: Overlain Spectra of CEF and TAZ in AUC Method (Area Under Curve Method).
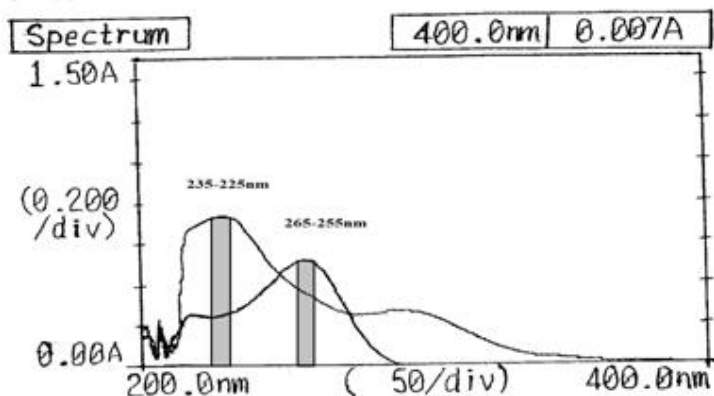

Fig.1 represents the overlain UV spectra of CEF and TAZ. CEF and TAZ exhibited linearity with absorbances in the range of 5-50 µg/mL and 2.5-15 µg/mL at their respective selected wavelengths. Co-efficient of correlation was found to be 0.9998 and 0.9982 for CEF and TAZ respectively. The optical characteristics and regression values for the calibration curves are presented in Table 1. For simultaneous estimation of CEF and TAZ, mixed standards containing CEF and TAZ in a concentration ratio of 8:1 µg/mL each were prepared by appropriate dilution of the standard stock solutions with 0.1M KOH. The areas of the mixed standard solutions were measured at the selected wavelengths. A set of two simultaneous equations were established using the mean absorptivity coefficients of CEF and TAZ at the selected wavelength intervals.

A1 = 393.025 C_{CEF} + 217.42 C_{TAZ} ......(i) at 235- 225nm
(λ1- λ2)
A2 = 180.4 C_{CEF} +595.2 C_{TAZ} ...... (ii) at 265-255 nm
(λ3- λ4)

Where, 393.025 and 180.4 are mean absorbtivity values of CEFO at (λ1- λ2) and (λ3- λ4) respectively.
217.42 and 595.2 are mean absorbtivity values of TAZ at (λ1- λ2) and (λ3- λ4) respectively.
A1 and A2 are the absorbance of mixed standards at (λ1- λ2) and (λ3- λ4) respectively.
C_{CEF} and C_{TAZ} are concentrations of CEF and TAZ.
The concentration of C_{CEF} and C_{TAZ} in mixed standard and injection formulation can be obtained by solving equations (i) and (ii).

Method B:
Absorbance Ratio or Q - Analysis Method
From the overlain spectrum of CEF and TAZ, two wavelengths were selected; one at 230nm λmax of CEF and other is 247nm, isoabsorptive point for both the drugs as showed in Fig.2.

Fig.2: Overlain Spectra of CEF and TAZ in Q analysis Method (Absorbance Ratio Method).
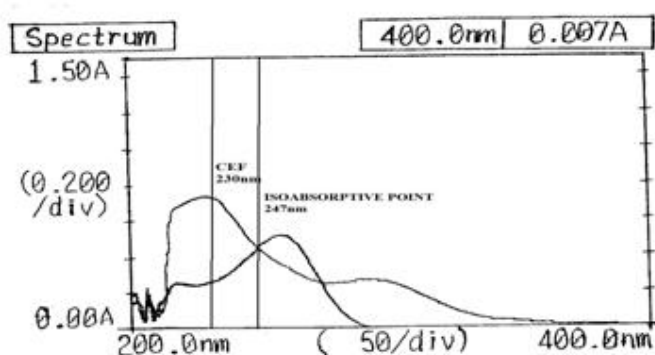
The standard and sample solutions were prepared in the same manner as in the previous method and absorbance measured at 230nm and 247nm. The drugs showed linearity in the concentration ranges of 5-50µg/mL, 2.5-15 µg/mL with regression coefficient (r^2) values of 0.9978, 0.9958 for CEF and TAZ respectively. Six mixed standards in ratio of 8:1 µg/mL showing linearity within the Beer's concentration range of CEF and TAZ were prepared by appropriate dilution of standard stock solutions (100µg/mL). The method employs Q values; the concentrations of drugs in sample solution were determined by using the following equations.

$$CCEF = \frac{Q0-Q2}{Q1-Q2} * \frac{A}{a1}$$ .... (iii)

$$CTAZ = \frac{Q0-q1}{Q2-q1} * \frac{A}{a2}$$ .... (iv)

Where,

Q_0= Absorbance of sample at 230 nm
Absorbance of sample at 247 nm

$Q1$= Absorptivity of CEF at 230nm
Absorptivity of CEF at 247 nm

$Q2$= Absorptivity of TAZ at 230nm
Absorptivity of TAZ at 247 nm

A = Absorbance of sample at isoabsorptive point
a1 and a2 absorptivities of CEF and TAZ respectively at isoabsorptivity point.

Assay of Injection Formulation:
Powder equivalent to 40 mg of CEF and 5 mg of TAZ was weighed and dissolved in 100 mL 0.1M KOH 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 TAZ. 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 AUC Method and equations (iii) and (iv) for the Q-Analysis method.

Validation:
The proposed methods were validated according to ICH Q2B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for the analyte[12].

Accuracy:
To ascertain the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%).

Linearity:
The linearity of measurement was evaluated by analyzing different concentration of the standard solution of CEF and TAZ. For AUC method and Q analysis, the Beer-Lambert's concentration range was found to be 5-50 µg/mL for CEF and 2.5-15 µg/mL for TAZ.

Precision:
Precision was studied to find out intra and inter-day variations in the test method of CEF and TAZ. Calibration curves prepared in medium were run in triplicate in same day and for three days. %RSD (relative standard deviation) were calculated which should be less than 2 %. The results are tabulated in the Tables.

The results of statistical validation data are given in table I. The results of the analysis and statistical validation data of recovery study of the injection formulation are given in table II and table III.

TABLE I: OPTICAL CHARACTERISTICS AND VALIDATION DATA OF CEF AND TAZ.

Parameters	CEF		TAZ	
	Method-A	Method-B	Method-A	Method-B
Working wavelengths	235-225nm	230 and 247nm	265-255nm	230 and 247nm
Beer-Lamberts Law range (µg/mL)	5-50	5-50	2.5-15	2.5-15
Precision*				
Interday (%RSD)				
Intraday (%RSD)	0.5218	0.3490	0.6613	0.2008
LOD (µg/mL)*	0.7051	0.1962	0.2688	0.0913
LOQ (µg/mL)*	0.6340	0.3117	0.2510	0.1752
Regression Values:	0.9172	0.7691	0.4702	0.2209
I. Slope*				
II. Correlation Coefficient (r^2)*	0.0378	0.0274	0.0398	0.0212
	0.9998	0.9982	0.9978	0.9958

*Denotes average of six estimations.
Method A – AUC Method (Area Under Curve Method)
Method B – Q-Analysis Method (Absorbance Ratio Method).

Table II: 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	993.9	99.39	0.2763	0.64075
	B	1000	998.2	99.82	0.3910	0.9775
TAZ	A	125	124.17	99.34	0.05317	1.0634
	B	125	125.31	100.25	0.03847	0.7694

The % drug obtained and % recovery value are mean of six determinations.
S.D.* = Standard deviation, n= 6, RSD= Relative standard deviation.
Injection Formulation, Magnova, manufactured by Lyka Labs Limited, Ankaleshwar; Marketed by LUPIN Limited. Mumbai, India.

TABLE III: STATISTICAL VALIDATION OF RECOVERY STUDIES

Level of % Recovery	Method	% Recovery*		% R.S.D.*	
		CEF	TAZ	CEF	TAZ
80	A	99.24	99.51	0.726	1.052
	B	99.68	99.56	0.985	0.873
100	A	100.27	99.43	0.0918	1.219
	B	99.95	99.77	0.532	0.531
120	A	99.17	100.62	0.392	0.726
	B	100.08	100.49	0.0735	0.418

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

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 99.57% and 99.79% formulation by the developed methods were 99.78% and 99.65% respectively (Table 2). The mean % recoveries of CEF and TAZ were found to be 99.79% and 99.59 % 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 TAZ are available in combined pharmaceutical dosage form for the treatment of lower respiratory tract infections, urinary tract infections, skin infections, bacterial meningitis, pneumonia, pyelonephritis, pediatric infections, etc. Here, two simple UV spectrophotometric methods (AUC Method (Area Under Curve Method), Q-Analysis Method (Absorbance Ratio 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 TAZ in both bulk and injection dosage form.

List of symbols and Abbreviations:
1. %   : Percent
2. nm   : Nanometer
3. μg/mL   : Microgram Per Mililitre
4. UV   : Ultraviolet
5. HPLC   : High Performance Liquid Chromatography
6. HPTLC   : High Performance Thin Layer Chromatography
7. CEF   : Cefepime
8. TAZ   : Tazobactam
9. KOH   : Potassium Hydroxide
10. ICH   : International Conference on Hormonization
11. SD   : Standard Deviation
12. RSD   : Relative Standard Deviation
13. LOD   : Limit of Detection
14. LOQ   : Limit of Quantitation

REFERENCES

1. Indian Pharmacopoiea, Vol. 2, Govt. Of India, Ministry of Health and Family Welfare, The Indian Pharmacopoiea Commission, Ghaziabad; 2010, pg. 1008.
2. British Pharmacopoeia, Vol. 1, The Department of Health, British Pharmacopoeia Commission, London; 2009.
3. United States Pharmacopoeia, United States Pharmacopoeial Convention. Inc, Rockville, MD, 2004, p.1621.
4. Rodenas, V., A. Parra, J. Garcia-Villanova and M.D. Gomez, 1995. Simultaneous determination of cefepime and L-arginine in injections by second-derivative spectrophotometry. J. Pharm. Biomed. Anal., 13: 1095-1099.
5. Elkhaili, H., L. Linger, H. Monteil and F. Jehl, 1997. High-performance liquid chromatographic assay for cefepime in serum. J. Chromatogr. B Biomed. Sci. Appl., 690: 181-188.
6. Patel S.A., Patel N.M., Patel M.M., Spectrophotometric estimation of Cefotaxime and Cefetriaxone in pharmaceutical dosage forms, Indian J .Pharm .Sci ., 2006, 68, 101-103.
7. Haginaka, J., J. Wakai, H. Yasuda, T. Uno and T. Nakagawa, 1984. Spectrophotometric determination of sulbactam by reaction with 1,2,4-triazole. Analytical, 109: 1057-1059.
8. Guillaume, Y., E. Peyrin and C. Guinchard, 1995. Rapid determination of sulbactam and tazobactam in human serum by high-performance liquid chromatography. J. Chromatorgr. B Biomed. Applied, 665: 363-371.
9. Mahgoub H., Aly F. A., UV determination of ampicillin sodium and Tazobactam in two component mixture, J .Pharm. Biomed. Anal., 1998, 8, 1273-1278.
10. Wang P., Yang J., A liquid chromatographic method for simultaneous determination of amoxicillin sodium and Tazobactam in combination formulation, J. Pharm. Biomed. Anal., 2004, 36, 565- 569.
11. Sanders, Jr. W., J. Tanney and R. Kessler, 1996. Efficacy of cefepime in the treatment of infections due to multiply resistant Enterobacter species. Clin. Infect. Dis., 23: 454-461.
12. ICH, Q2 (R1), Harmonised tripartite guideline,Validation of analytical procedures: text and methodology International Conference on Harmonization ICH, Geneva, Nov 2005.