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Spectrophotometric absorbance correction method for the estimation of Tazobactam and Cefepime in combined tablet dosage forms

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ABSTRACT

A new, simple, precise, accurate and sensitive UV - Spectrophotometric absorption correction method has been developed for simultaneous determination of Tazobactam and Cefepimein combined tablet dosage form using 0.1 N NaOH as solvent. Absorbance correction method was based on the property of additivity of absorbances. The wavelengths selected for the absorption correction method were 259 nm and 306 nm. At 306 nm, Cefepime showed some absorbance while Tazobactam showed zero absorbance. Both the drugs gave absorbance at 259 nm. The method involved solving of an equation based on measurement of absorbance at two wavelengths 259 and 306 nm. The method was validated statistically. The determinations were made at 259 nm for Tazobactam and Cefepime and 306 nm for Cefepime over the concentration range of 3-18 µg/ml for Tazobactam and 10-50µg/ml for Cefepime with mean recovery of 100.34 ± 0.73 % and 99.89 ± 0.52 f or Tazobactam and Cefepime, respectively by absorbance correction method. The precision for intra-day and inter-day of the method were found to be within the limits (RSD <2%). This method was found to be precise, accurate, simple, sensitive, reproducible and economical and can be applicable for the simultaneous determination of Tazobactamand Cefepime in combined dosage form.

Key words: Cefepime, Tazobactam, Absorbance correction method, Method validation

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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]. Cefepime (CEF) is a well-known fourth generation broad spectrum injectable, semi synthetic cephalosporin. It is very much similar to the third-generation Cephalosporins structurally, except that it has a N-methylpyrrolidinium at the 3-position, rendering it a zwitterion[4,5]. Like other fourth generation cephalosporins, cefepime demonstrates good activity against gram-negative organisms such as *Pseudomonas aeruginosa* and gram-positive organisms such as *Staphylococcus aureus*[6]. It is indicated for respiratory tract infections, skin and soft tissue infections, urinary tract infections and febrile neutropenia[6, 7]. Literature survey reveals several spectroscopic[8] HPLC[9] and HPTLC methods for the estimation of CEF individually as well as in combination with other drugs[10].

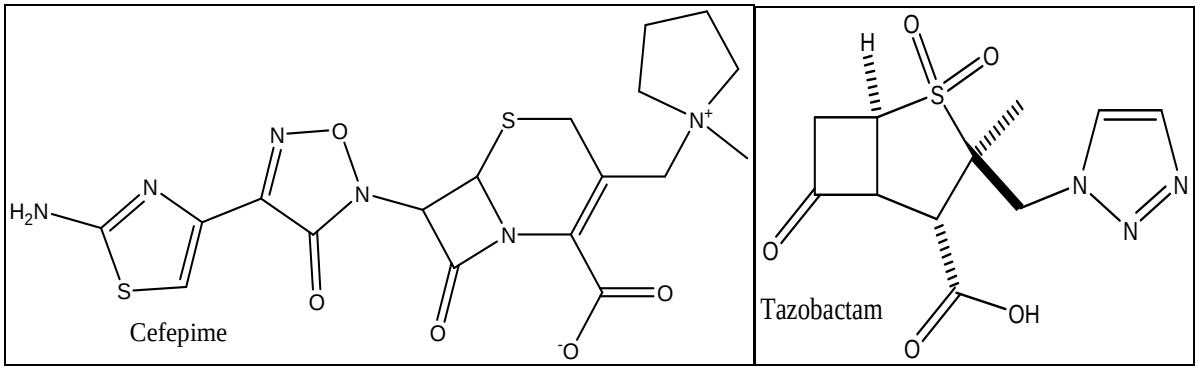


Fig. 1: Chemical structure of (a) Cefepime and (b) Tazobactam

Tazobactam (TAZ) is chemically (2S,3S,5R)-3-methyl-4,4,7-trioxo-3-(1H-1,2,3-triazol-1-ylmethyl)-4λ^6-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide sodium salt[11]. It is a Antipseudomonal penicillins (beta-lactamase inhibitor with antibacterial properties)[12]. It belongs to a class of penicillanic acid sulfones which acts by inhibiting bacterial β-lactamases. Infection caused by β-lactamase producing bacterial strains has recently become a major problem in hospitals. Several β-lactamase inhibitors have been developed against the target enzyme[13]. When they are combined with some penicillins or cephalosporins, the mixed ingredients have been shown to be effective against various β-lactamase producing bacteria both in vitro and in vivo[14-17]. Tazobactam sodium is official in USP[18]. Literature survey reveals UV spectroscopic[19] and HPLC[20] methods for the estimation of TAZ individually as well as in combination with other drugs[21,22].

CEF and TAZ are formulated together in the form of sterile powder for injection for the treatment of lower respiratory tract infections, skin infections, urinary tract infection, gynecological infection and post-operative infection[23].

An exhaustive review of the various analytical methods available for these drugs have been carried out by the authors,no spectrophotometric method is available for the simultaneous analysis of CEF and TAZ. Present paper describes accurate, reproducible, simple, rapid, and economical method for the simultaneous determination of CEF and TAZ in parenteral formulations using Absorption Correction Method.

EXPERIMENTAL SECTION

Apparatus
UV/Visible Spectrophotometer: SICAN-2301.
Analytical Balance: Sartorious BSA223S-CW.
Magnetic Stirrer: REMI 1MLH, Remi Laboratories Limited.

Material
Active pharmaceutical ingredient of Tazobactamwas supplied by Swati Chemicals, Ahmedabad, Gujarat.

Active pharmaceutical ingredient of Cefepime was supplied by Balsam Life Sciences & Technologies Pvt Ltd, Kalyan, Thane (India).

Marketed formulation
MAGNOVA 1 mg injection vials are from Lupin Ltd.

Reagents
0.1 N NaOH prepared from double distilled water obtained using Millipore Filter Assembly was used throughout the analysis.

Methodology

Accurately weighed quantities (100 mg) of CEF and TAZ were taken in 100ml standard flasks, dissolved separately by adding 50 ml 0.1 N NaOH and volumes were made up (1000 µg/ml). These solutions were used as working standards. Aliquot portions of stock solutions of TAZ and CEF were diluted appropriately with 0.1 N NaOH to obtain concentration 3 µg/ml of TAZ and 24 µg/ml of CEF. The working standard solutions were scanned from 200 to 400 nm to select the wavelengths for estimation. From the overlain spectrum shown in Fig.1, the wavelength selected for estimation of CEF was 306 nm, where TAZ has no significant absorbance and for TAZ it was 259 nm, where absorbance of TAZ is corrected. Different binary mixture solutions of TAZ and CEF were then run in entire range from 200 to 400 nm. The drugs obey Beer's law in the concentration range of 3 to 18 µg/ml and 10 to 50 µg/ml for TAZ & CEF respectively. All the optical characteristics were tabulated in Table-1.

Quantitative estimation of these drugs were calculated using following equations

$A = abc$

$Cx = A1 / ab$

$Cx = A1 / ax1 * b$…… . (1)

$A2 = A\ cef + A\ taz$

$A2 = (ay2 * cy * b) + (ax2 * cx * b)$

$A2 = (ay2 * cy) + (ax2 * cx)$

$Cy = [A2 - (ax2 * cx)] / ay2$……. (2)

where *A*1, *A*2 are absorbance of mixture at 306 nm (λ1) and 259 nm (λ2), respectively, *ax*1 and *ax*2 are absorptivities of CEF at λ1 and λ2, respectively, *ay*1 and *ay*2 are absorptivities of TAZ at λ1 and λ2, respectively, *c*x and *c*y are concentrations of CEF and TAZ, respectively.

Preparation of standard stock solutions:

An accurately weighed quantity of CEF (250 mg) and TAZ (31.25 mg) were transferred to separate 250 ml volumetric flask and dissolved and diluted to the mark with methanol to obtain standard solution having concentration of CEF (48µg/ml) and TAZ (6µg/ml).

Preparation of sample solution:

Twenty Tablets were weighed and powdered. The powder equivalent to 250 mg of CEF and 31.25 mg of TAZ was transferred to a 250 ml volumetric flask, added with 0.1N NaOH and stirred on magnetic stirrer for 60 min. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to the mark with 0.1 N NaOH. The above solution was suitably diluted with methanol to get a final concentration of 48 µg/ml of CEF and 6µg/ml of TAZ.

Validation of proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[24].

Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 3-18µg/ml for TAZ and 12-60 µg/ml for CEF. Accurately measured standard stock solutions of each TAZ (3, 6, 9, 12, 15, 18) and CEF (12, 24, 36, 48 and 60µg/ml) were transferred to a series of 10 ml volumetric flask separately. The absorbance of solutions were measured at 259 nm and 306 nm. The calibration curves were constructed by plotting absorbance versus concentration and the regression equations were calculated

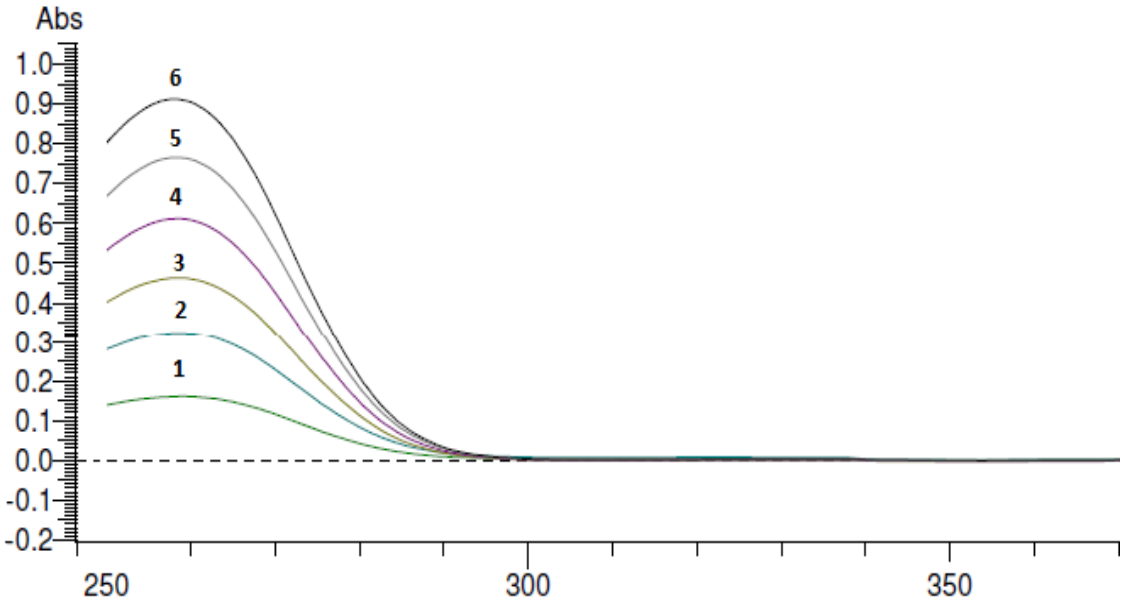


Fig. 2: Overlay spectra of Tazobactam (3-18 µg/ml)

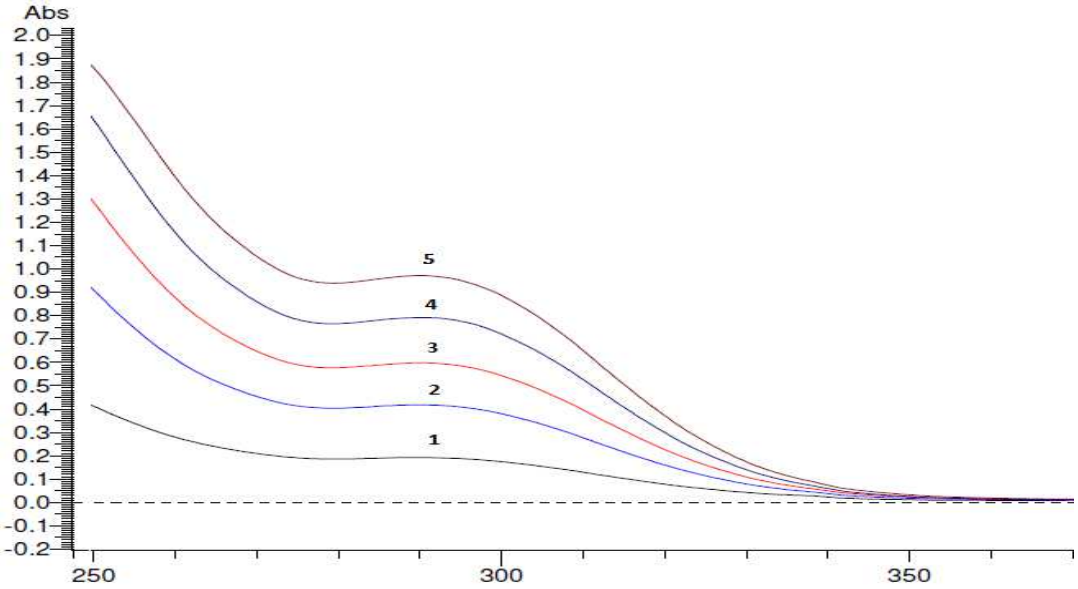
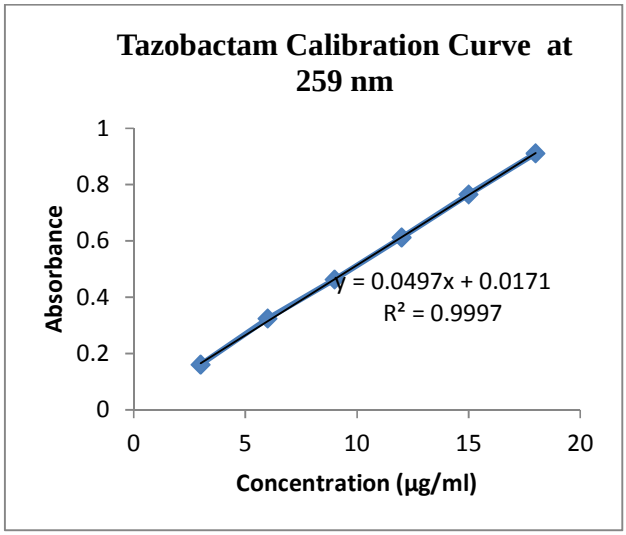


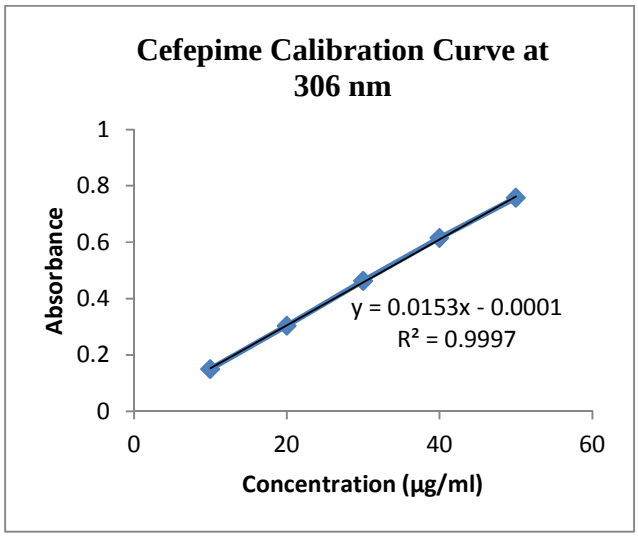
Fig.3: Overlay spectra of Cefepime (12-60 µg/ml)

Table 1: Data of optical characteristics

Parameters	Observed Value	
	Drugs	
	Tazobactam	**Cefepime**
Beer's Law Limit (µg/ml)	3-18 µg/ml	12-60 µg/ml
Correlation Coefficient (R^2)	0.9997	0.9997
Regression Equation (y=mx+c)	y = 0.0497x + 0.0171	y = 0.0153x - 0.0001
Slope	0.0497	0.0153
Intercept	0.0171	-0.0001



(a)



(b)

Fig.4: Linearity graph for (a) Tazobactam and (b) Cefepime

Precision

Intraday

Mixed standard solutions containing 3, 4.5, 6µg/ml TAZ and 24,36,48µg/ml of CEF was analyzed three times on the same day. Measure the solution at 306 nm (A1) and 259 nm (A2). The results were reported in terms of relative standard deviation (Table 2).

Interday

Mixed standard solution containing 3, 4.5, 6 µg/ml TAZ and 24,36,48µg/ml of CEF was analyzed on 3 different days. Measure the solution at 306 nm (A1) and 259 nm (A2). The results were reported in terms of relative standard deviation (Table 2).

Table 2: Precision Studies
Intraday analysis of formulation

Drug	Sampling Time	Concentration (µg/ml) taken	Concentration found (µg/ml)	%age obtained	S.D.	%R.S.D.
	9:00 AM	24	24.10	100.43	0.18	0.73
Cefepime	1:00 AM	36	35.86	99.60	0.27	0.75
	5:00 PM	48	48.02	100.05	0.44	0.92
	9:00 AM	3	20.07	100.36	0.18	0.87
Tazobactam	1:00 AM	4.5	30.08	100.26	0.18	0.60
	5:00 PM	6	39.81	99.52	0.48	1.22

Interday analysis of formulation

Drug	Sample No.	Concentration (µg/ml) taken	Concentration found (µg/ml)	%age obtained	S.D.	%R.S.D.
	Day 1	24	24.06	100.23	0.18	0.76
Cefepime	Day 2	36	35.80	99.44	0.41	1.15
	Day 3	48	48.06	100.13	0.74	1.54
	Day 1	3	3.00	100.13	0.02	0.65
Tazobactam	Day 2	4.5	4.49	99.87	0.05	1.18
	Day 3	6	6.01	100.10	0.09	1.48

Specificity

Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix. Commonly used excipients in tablet preparation were spiked in a preweight quantity of drug and then absorbance was measured and calculation done to determine quantity of drugs.

Accuracy (Recovery Studies)

The accuracy of the method was determined by calculating recoveries of TAZ and CEF by the standard addition method. Accuracy is performed at three levels 80, 100 and 120%. Known amount of standard solutions of TAZ (2.4, 3 and 3.6) and CEF (19.2, 24 and 28.8 µg/ml) were added to a pre-quantified test solution of TAZ (3 µg/mL) and CEF (24 µg/mL). Absorbance of solution was measured at selected wavelength for TAZ and CEF.The amount of TAZ and CEF was calculated at each level by absorbance correction equation method and percentage recoveries were computed (Table 3).

Table 3: Recovery Studies
Accuracy (Recovery Studies of Cefepime)

Drug (level of % recovery)	Sample No	Amount Present, B (µg/ml)	Amount added, C (µg/ml)	Amount found, A (µg/ml)	Amount recovered (A-B) (µg/ml)	% Recovered [(A-B)/C]*100 (µg/ml)	S.D.	%RSD
Cefepime (80%)	1	24	19.2	43.19	19.19	99.96	0.13	0.13
	2	24	19.2	43.23	19.23	100.16		
	3	24	19.2	43.19	19.19	99.93		
					Mean	100.02		
Cefepime (100%)	1	24	24	48.13	24.13	100.55	0.53	0.52
	2	24	24	47.96	23.96	99.85		
	3	24	24	48.21	24.21	100.88		
					Mean	100.43		
Cefepime (120%)	1	24	28.8	52.34	28.34	98.40	0.91	0.92
	2	24	28.8	52.53	28.53	99.06		
	3	24	28.8	52.86	28.86	100.21		
					Mean	99.22		

Accuracy (Recovery Studies of Tazobactam)

Drug (level of % recovery)	Sample No	Amount Present, B (µg/ml)	Amount added, C (µg/ml)	Amount found, A (µg/ml)	Amount recovered (A-B) (µg/ml)	% Recovered [(A-B)/C]*100 (µg/ml)	S.D.	%RSD
Tazobactam (80%)	1	3	2.4	5.43	2.43	101.25	0.42	0.41
	2	3	2.4	5.44	2.44	101.67		
	3	3	2.4	5.42	2.42	100.83		
					Mean	101.25		
Tazobactam (100%)	1	3	3	6.02	3.02	100.67	0.88	0.88
	2	3	3	5.98	2.98	99.33		
	3	3	3	5.97	2.97	99.00		
					Mean	99.67		
Tazobactam (120%)	1	3	3.6	6.64	3.64	101.11	0.89	0.89
	2	3	3.6	6.59	3.59	99.72		
	3	3	3.6	6.58	3.58	99.44		
					Mean	100.09		

Limit of detection and limit of quantitation

Limit of detection is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value and limit of quantitation is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$LOD=3.3\sigma/S$
$LOQ=10\sigma/S$

where, σ = the standard deviation of the response andS = slope of the calibration curve.

Analysis TAZ and CEF in Combined Dosage Forms

Pharmaceutical formulation of TAZ and CEF was purchased from local pharmacy. The responses of formulations were measured at 259 nm and 306 nm for CEF and TAZ, respectively by absorbance correction method as described above. The amounts of TAZ and CEF present in sample solution were determined by fitting the responses into the regression equation for TAZ and CEF in the method using Equation 1 and 2 (Fig. 5).

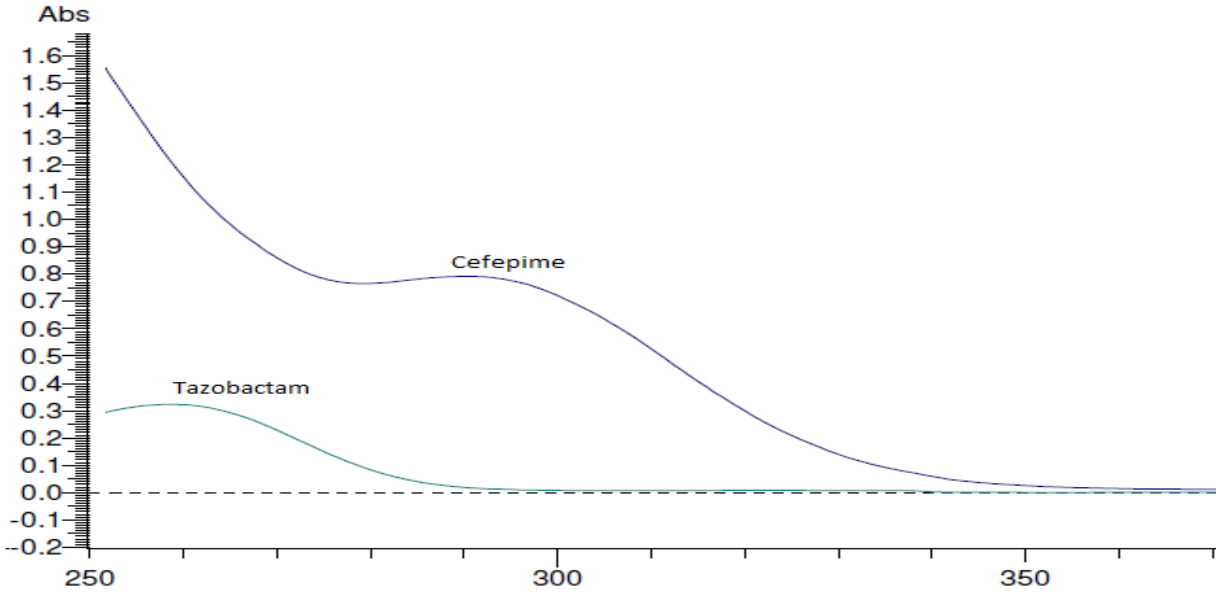


Fig. 5: Overlay Spectra of Tazobactam (6 µg/ml) and Cefepime (48 µg/ml)

RESULTS AND DISCUSSION

Absorbance correction method
The utility of dual wavelength data processing program is its ability to calculate unknown concentration of component of interest in a mixture containing an interfering component. For elimination of the effects of an interfering component, two specific wavelengths were chosen.

1. First wavelength λ1 at which minimum absorbance of CEF was observed and there was no interference of TAZ at this wavelength (306 nm).

2. Second wavelength λ2 was the wavelengths absorption maxima of TAZ and also CEF gives some absorbance at this wavelength (259 nm). To remove the interference of CEF to the absorbance at 259.0 nm (λ2), another wavelength 306 nm (λ1) was found out at which the absorbance of TAZ was zero. These two selected wavelengths were employed to determine the concentration of TAZ from the mixture of TAZ and CEF (Fig. 1). The difference in absorbance at these two wavelengths (A259 – A306) cancels out the contribution of absorbance of CEF in mixture (Fig. 5).

Validation data of the proposed methods
Linearity - Linear correlation was obtained between absorbance and concentration of TAZ and CEF in the range of 3-18 and10-50µg/ml respectively. The linearity of the calibration curves was validated by the high value of correlation coefficients of regression (Table 1).

Precision - The low RSD values of interday (0.65-1.48 and 0.76 - 1.54 %) and intraday (0.97 - 1.63 % and 0.73 - 0.92 %) variations for TAZ and CEF, respectively reveal that the proposed method is precise (Table 2).

Accuracy - The recovery experiments were carried out by the standard addition method. The mean recovery obtained was 100.34 ± 0.73 % and 99.89 ± 0.52 for TAZ and CEF, respectively (Table 3). The high values indicate that the method is accurate.

LOD and LOQ –
LOD for TAZ and CEF were found to be 0.92µg/ml and 0.98µg/ml, respectively whereas LOQ for TAZ and CEF were found to be 2.79µg/ml and 2.97 µg/ml, respectively. The data shows that the method is sensitive for the determination of TAZ and CEF, in the given concentration range.

Assay of the pharmaceutical formulation
The proposed validated methods were successfully applied to determine TAZ and CEF in their marketed dosage forms. The results obtained for TAZ and CEF were comparable to the corresponding labeled amounts (Table 4).

Table 4: Analysis of tablet formulation

Brand	Drug	Labelled Claim (mg/tab)	Amount Found (mg/tab)	%Purity	SD	%RSD
Magnova	Cefepime	1000	1000.85	100.08	1.69	0.17
(Lupin Ltd.)	Tazobactam	125	125.24	100.19	0.52	0.42
Celrim TZ	Cefepime	1000	1000.86	100.09	1.02	0.10
(Biocon Ltd.)	Tazobactam	125	125.17	100.14	0.38	0.30

CONCLUSION

The results of the analysis of pharmaceutical formulation by the proposed method are highly reproducible and reliable and are in good agreement with the label claim of the drug. The additives usually present in the pharmaceutical formulations of the assayed samples did not interfere with determination of TAZ and CEF. The methods can be routinely used for the analysis of the TAZ and CEF in combined dosage form.

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