Research Article:

Development and Validation of Spectrophotometric Methods for Simultaneous Estimation of Cefixime Trihydrate and Linezolid in Tablet Dosage Form

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Abstract:

A new, simple, rapid and novel spectrophotometric method has been developed for simultaneous estimation of cefixime trihydrate and linezolid. For this, simultaneous equation method is used. The method involved measurement of absorbance at two wavelengths, 250 nm and 286 nm, λ_{max} of linezolid and cefixime trihydrate respectively. This method obeys Beer's law in the employed concentration ranges of 2-12 µg/ml for both cefixime trihydrateand linezolid. The proposed method is recommended for routine analysis since it is rapid, simple, accurate, specific and also sensitive. This paper describes the development and validation of UV spectroscopic method for simultaneous estimation of cefixime trihydrate and linezolid in Tablet dosage form.

Keywords: *cefiximetrihydrate, linezolid, simultaneous estimation method, validation*

1. Introduction

Chemically cefixime (Fig. 1) is (6R, 7R)-7-[2-(2-amino-4- thiazolyl) glyoxylamido]- 8-oxo-3-vinyl-5-1-azabicyclo [4.2.0] oct-2- ene-2-carboxylicacid, 7-9z)-[o carboxymethyl)-oxime] trihydrate. Cefixime is a third generation cephalosporin antibiotic [1,2]. It is under the category of β-Lactam antibiotics/cell wall inhibitor. It acts by inhibiting an enzyme transpeptidase involved in the building of bacterial cell walls [3]. It is used in lower respiratory tract infections [4,5,6], acute urinary tract infections [6,7], biliary tract infections [8], sinusitis [9], acute otitis media [10], peptic ulcer [11].

Linezolid is a synthetic antibacterial agent of the oxazolidinone class. The chemical name for linezolid (Fig. 2) is (S)-N-[[3-[3-Fluoro-4-(4- morpholinyl) phenyl]-2-oxo-5-oxazolidinyl] methyl]-acetamide[2,12]. Linezolid is a member of a new structural class of antibiotics, oxazolidinones. The oxazolidinones have a good activity against Gram-positive bacteria [13,14]. They act uniquely by inhibiting the formation of protein synthesis initiation in Gram-positive

bacteria [15]. Cross-resistance with existing antibiotics has not been demonstrated till date. Linezolid is active after oral or intravenous administration. Linezolid is expected to increase the treatment options for severe infections due to Gram positive bacteria, particularly resistant infections (e.g. *Methicillin-resistant Staphylococcus aureus* (MRSA) and Vancomycin-resistant Enterococci (VRE)) [16,17]. Appropriate use of linezolid is essential to minimize the risk of resistance development in Gram positive bacteria. The availability of both parenteral and oral formulations provides the opportunity to transfer appropriate patients to an oral formulation. As per literature survey, Cefixime can be estimated by spectrophotometrically [18-27] and by HPLC [27-31] individually or with other drugs in bulk drugs and in human plasma, while linezolid can be estimated by spectrophotometrically [32], HPLC [1,33] and HPTLC [34] in combination with other drugs. However, there is no analytical method has been reported for simultaneous estimation of Cefixime trihydrate and linezolid in pharmaceutical dosage forms. Therefore, in the present research work, our aim is to develop a novel, simple, accurate, sensitive, reproducible and economical analytical method to estimate Cefixime trihydrate and linezolid in their combined dosage form in routine analysis.

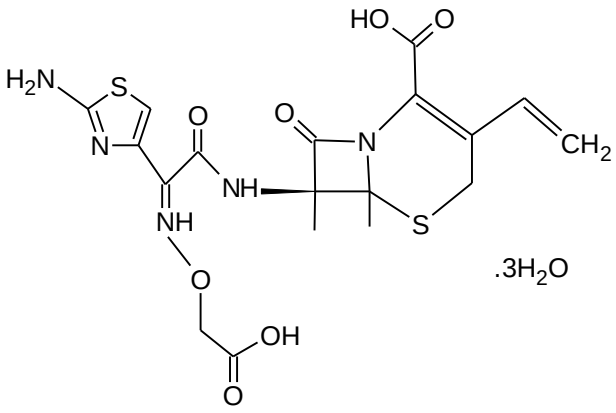

FIGURE 1: Chemical Structure of cefixime Trihydrate.

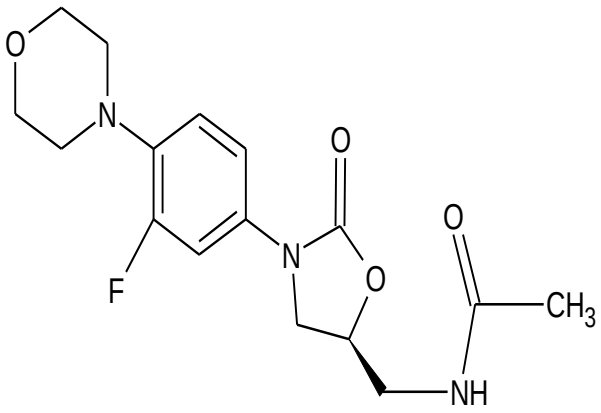

FIGURE 2: Chemical Structure of linezolid.

2. Materials and Methods

*2.1 Apparatus and Instruments.*Spectrophotometric measurements were made onUV-visible double beamspectrophotometer (UV-1800, Shimadzu Corp., Japan) with spectral bandwidth of 2 nm and 10 mm matched quartz cellsfor development of analytical method over the range

of 200-400 nm. The drugs and chemicals were weighed on Anamed analytical digital weighing balance (AA – 2200, Anamed).

*2.2 Chemicals and Reagents.*Standard bulk sample of cefixime trihydrate and linezolid were pursued as a gift sample from Cipla Ltd., Mumbai, India and Macleoids Ltd., Mumbai, India respectively.Sodium hydroxide pellets (S. D. fine chemicals Ltd.,India) was used to obtain 0.2 N NaOH as a solvent.

*2.3 Marketed Formulation.*Marketed tablet formulation (Lizokef tablet, Macleoids Ltd., Mumbai, India) containing labeled amount of CEF 200 mg and LNZ 600 mg was used as sample; purchased from local pharmacy.

2.4 Selection of Common Solvent. Sodium Hydroxide (NaOH) of analytical reagent grade was selected as a common solvent for developing spectral characteristics of both drugs. The selection was made after assessing the solubility of both drugs in different solvents.

*2.5 Preparation of Standard Solutions.*Standard stock solutions were prepared byaccurately weighing 10 mg each ofCEF and LNZ in 100 ml 0.2 N NaOH solution to make stock solution of concentration 100 µg/ml individually.Suitable aliquots of these stock solutions were taken to prepare standard solutions containing 2-12 µg/ml of each of CEF and LNZ.

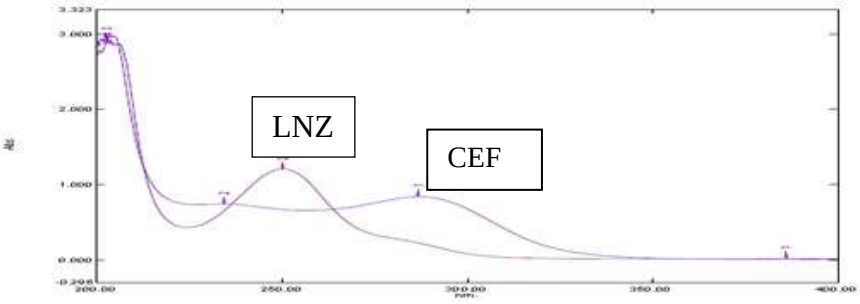

FIGURE 3: Overlain spectrum of the CEF and LNZ

2.6 Selection of Analytical Wavelength. For the development of simultaneous equation method, the wavelength maxima of both the drugs are required. Appropriate dilutions were done for each drug from the standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. LNZ and CEF showed absorbance maxima at 250 nm and at 286 nm respectively(Figure 3).

2.7 Calibration Curves forCEF and LNZ. Appropriate aliquots of CEF and LNZ working standard solutions were taken in different 10mL volumetric flasks and diluted up to the mark with 0.2 N NaOH to obtain final concentrations of 2, 4, 6, 8, 10, 12 and 15 μg/mL of CEF and LNZ respectively. Spectra of the solutions were scanned between 400- 200nm. The absorbance of solutions was measured at 286 nm and 250 nm against 0.2 N NaOH as blank, and the absorbance versus concentration was plotted. The straight line equations for both drugs at two wavelengths were determined, and the values of absorbance were obtained.

TABLE 1: Standard calibration for CEF and LNZ

Sr. No.	CefiximeTrihydrate		Linezolid	
	Conc. (µg/ml)	Abs. at 286 nm	Conc. (µg/ml)	Abs. at 250 nm
1.	2	0.022	2	0.015
2.	4	0.041	4	0.03
3.	6	0.061	6	0.046
4.	8	0.082	8	0.061
5.	10	0.099	10	0.073
6.	12	0.121	12	0.089

2.8 Validation. The method was validated for linearity, precision, LOD and LOQ by following procedure.

2.6.1 Linearity. Both drugs followed the Beer-Lamberts law in the range of 2-12 µg/ml. Absorbances for these solutions were measured at 250 nm and 286 nm for LNZ and CEF respectively (Table 1). Calibration curve are shown in Figure 4 & 5; Regression coefficient are 0.999 and 0.999 for CEF and LNZ respectively. Table 2 summaries the optical characteristics of both the drugs.

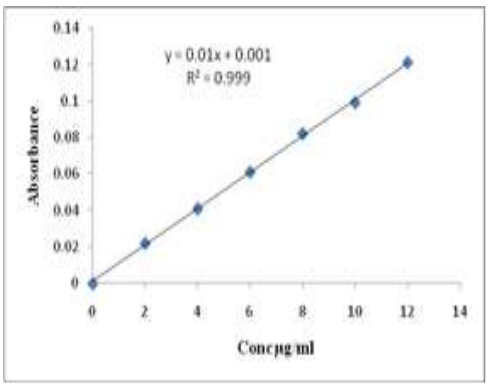

FIGURE 4: Calibration curve of CEF

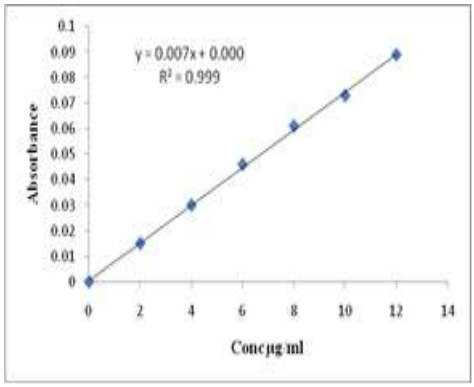

FIGURE 5: Calibration curve of LNZ

TABLE 2: Optical characteristics and other parameters

Parameters	CEF	LNZ
Working wavelength (nm)	286	250
Linearity range (µg/ml)	2-12	2-12
Limit of detection (µg/ml)	0.2883	0.4243
Limit of quantitation (µg/ml)	0.9513	1.4001
Slope	0.0103	0.0073
Intercept	0.0017	0.0008
Regression Coefficient	0.999	0.999

2.8.2 Precision. Precision of the method was verified by using stock solutions in the ratio of 1:3 containing 0.5 µg/ml of CEF and 1.5 µg/ml of LNZ. System repeatability was done by repeating the assay three times of six replicate dilutions of the same concentration after every four hours on the same day for intraday precision. Interday precision was carried out by performing the assay of six sample sets after 24 hours and 48 hours. The results of intermediate precision are given in Table 3.

TABLE3: Results of Precision study

Formulation	Parameter	Intra-day precision*	Inter-dayprecision*

CEF	% Mean	99.56	100.32
	SD	0.6984	0.6874
	% RSD	0.7014	0.6852
	% Mean	98.84	99.68
LNZ	SD	0.6385	0.6124
	% RSD	0.6460	0.6144

*Each value is a mean of six observations.

2.8.3 LOD and LOQ. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the standard deviation of responses (N) and slopes (S) of respective calibration curves using signal-to-noise ratio (Table 4)

$$LOD = 3.3 \times N/S$$

$$LOQ = 10 \times N/S$$

TABLE4: Results of LOD & LOQ

Parameters	**CEF**	**LNZ**
Limit of Detection (µg/ml)	0.2883	0.4243
Limit of Quantitation(µg/ml)	0.9513	1.4001

2.9 *Analysis of Marketed Formulations.* For analysis of tablet formulation, first twenty tablets were weighed accurately; the average weight was determined and then triturated to a fine powder. A quantity equivalent to 200 mg of CEF and 600 mg of LNZ was weighed and transferred to a 100 ml volumetric flask and sonicated for 30 min to dissolve the active ingredients with 50 ml of 0.2 N NaOH solution and the volume was made up to 100 ml with 0.2 N NaOH solution and filtered through Whatman filter paper no. 41 to give the stock solution containing 2000 µg/ml of CEF and 6000 µg/ml of LNZ.Various dilutions of the

tablet stock solutions were scanned and the absorbances of these solutions were measured at 286 nm and 250 nm respectively and the concentrations of the two drugs in the sample solutions were determined by simultaneous equation. The analysis procedure was repeated six times (Table 5).

TABLE 5: Results of Analysis of Marketed Formulations

Sr. no.	**Label Claim (mg/tab)**		**Amount Found (mg/tab)**		**% of Label Claim**	
	CEF	LNZ	CEF	LNZ	CEF	LNZ
1	200	600	198.089	600.103	99.04	100.01
2	200	600	201.084	599.441	100.54	99.90
3	200	600	198.213	597.664	99.10	99.61
4	200	600	201.006	599.342	100.50	99.89
5	200	600	199.861	598.985	99.93	99.83
6	200	600	201.465	600.29	100.73	100.04
			Mean*		99.97	99.88
			SD*		0.683	0.143
			% RSD*		0.683	0.143

*Denotes average of 6 determinations

Formulation: *Lizokef* (InnovaCapTab Pvt. Ltd., Solan, Himachal Pradesh, India)

2.10 Recovery study. Recovery studies were carried out at three levels i.e. 80, 100 and 120 % of the label claim of the Tablet formulation as per ICH guidelines. To perform recovery studies at 80 % of the test concentration, sample containing 200 mg of CEF and 600 mg LNZ was weighed and transferred to a 100 ml volumetric flask. To it 160 mg of standard CEF and 480 mg of standard LNZ was added, the mixture was mixed thoroughly. The contents were sonicated for 20 min with 0.2 N NaOH solutionto dissolve the active ingredients and filtered through Whatman filter paper no. 41. Similarly to perform recovery studies at 100 % of the test concentration, tablet powder containing 200 mg of CEF and 600 mg of LNZ was weighed. To it, 200 mg of standard CEF and 600 mg of standard LNZ was added and at 120 % level, 240

mg of standard CEF and 720 mg of standard LNZ was added to the tablet powder equivalent to 200 mg of CEF and 600 mg of LNZ. These contents were sonicated for 20 min with 0.2 N NaOH to dissolve the active ingredients and filtered through Whatman filter paper no. 41. From the stock solutions prepared, at each level suitable aliquots were pipetted out and diluted to 10 ml with 0.2 N NaOH and were analyzed as per the procedure for tablet formulations. The results of the recovery studies were also validated statistically (Table 6).

TABLE6: Results of Recovery studies

Level of recovery %	% mean*		Standard Deviation*		% Relative Standard Deviation*	
	CEF	LNZ	CEF	LNZ	CEF	LNZ
80	99.76	99.96	0.4804	0.1217	0.4814	0.1217
100	99.84	99.93	0.2086	0.0909	0.2089	0.0909
120	100.02	99.99	0.0448	0.0228	0.0448	0.0228

*Each value is the mean of three observations

3. Results and Discussion

The novel method for simultaneous estimation of CEF and LNZ was developed using 0.2 N NaOH as solvent. CEF and LNZ follows Beer-Lambert's law in range of 2-12 µg/ml. Commercial formulation containing CEF and LNZ were analyzed by proposed method. Mean assay values in *Lizokef* were found to be 99.97 and 99.88 for CEF and LNZ respectively. The accuracy of method was determined by recovery studies. Pure CEF and LNZ were added to the preanalyzed tablet powder at three different levels i.e. 80, 100 & 120% of labeled claims as per the ICH guidelines. Three replicate analyses were carried out at each level. The mean recovery was found to be 99.87 % and 99.96 % for CEF and LNZ in *Lizokef* samples respectively

indicating that the method has required accuracy and there was no interference by excipients present in tablets. The RSD value below 2% indicated that the method has required precision. The LOD and LOQ values of CEF and LNZ at 286 and 250 were found to be 0.2883and 0.9513 µg/ml and 0.4243 and 1.4001 µg/ml respectively.

4. Conclusion

Simultaneous equation method has been developed for theestimation of CEF and LNZ in their combined dosage form.The method was validated and found to be simple, sensitive,accurate, and precise. Simultaneous equation method is havingadvantage that it is simple, it requires less analysis time,and the cost of the analysis is less compared to chromatographicmethod. Hence, it can be successfully applied for routine estimation for CEF and LNZ in quality control laboratories.

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