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Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Cefixime and Linezolid in Combined Dosage Form

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ABSTRACT

Cefixime(CEF) is a cephalosporin class of antibiotic drug and Linezolid(LIN) is a oxazolidinone class of antibiotic drug. This combination of drug is useful in many bacterial diseases. Accurate, precise, rapid and economical method was developed for the simultaneous estimation of Cefixime(CEF) and Linezolid(LIN) in tablet dosage form. Method is based on the simultaneous equations and wavelengths selected for analysis were 289.0nm (λmax of Cefixime) and 257.0 nm (λmax of Linezolid) respectively, in methanol. The linearity was obtained in the concentration range of 5-40µg/ml for Cefixime and 10-30µg/ml for Linezolid. The correlation coefficient of Cefixime and Linezolid were found to be 0.9998 and 0.9998 respectively. The proposed procedure was successfully applied for the simultaneous determination of both drugs in commercial tablet preparation. The results of the analysis have been validated statistically and by recovery studies have confirmed the accuracy of proposed method.

KEYWORDS

Cefixime(CEF), Linezolid(LIN), Simultaneous equations, Ultra-Violet Spectrometric method (UV Spectrometric method).

INTRODUCTION

Cefixime (CEF) is an oral third generation cephalosporin class of antibiotic. Chemically, it is (6*R*, 7R)-7-{[2-(2-amino-1,3-thiazol-4-yl)-2(carboxymethoxy-imino)acetyl]amino}-3-ethenyl-8-oxo-5-thia-1 azabicyclo-[4.2.0]oct-2-ene-2 carboxylic acid[1,3], clinically used in the treatment of susceptible infections including gonorrhea, otitis media, pharyngitis, lower respiratory-tract infections such as bronchitis, and urinary-tract infections[1,3]. Linezolid (LIN) is first of the oxazolidinone class of antibiotic drug and chemically it is N-{[(5S)-3-[3-fluoro-4-(morpholin-4-yl)phenyl]-2-oxo-1,3-oxazolidin-5-yl]methyl}acetamide and it is also useful as Antibacterial Agents[2,3].

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Both the drugs are marketed as combined dose tablet formulation in the ratio of 200:600 mg CEF: LIN. Literature survey reveals that cefixime can be estimated by spectrophotometrically[4-13] and by HPLC[13-17] individually or with other drugs in bulk drugs and in human plasma, while Linezolid can be estimated by spectrophotometrically[22], HPLC[1,19] and HPTLC[18] in combination with other drugs. However, there is no analytical method has been reported for the estimation of CEF and LIN in a combined dosage formulation. Present work describes the Simultaneous equation method for simultaneous estimation of CEF and LIN in tablet formulation.

MATERIALS AND METHODS

Instrument

A double-beam Shimadzu-1800 UV- Visible spectrophotometer, with spectral bandwidth of 2 nm, wavelength accuracy ± 0.5 nm and a pair of

1-cm matched quartz cells was used to measure absorbance of the resulting solution.

Materials

Standard gift sample of Cefixime was provided by Mepro Pharmaceuticals Ltd, Dholka and Linezolid was povided by Cadila Healthcare Pvt. Ltd, Dabhasa. Combined dose Cefixime and Linezolid tablets were purchased from local market. Methanol (AR Grade) was used as solvent, procured from Loba Chemie, Mumbai.

Stock Solutions

Stock solution Standard stock solutions of CEF (100 µg/ml) and AZI (100 µg/ml) were prepared in methanol and used for the analysis.

Spectral Characteristics of CEF and LIN

Solutions of CEF and LIN (10µg/ml, each), were prepared separately by appropriate dilution of standard stock solution. Both the solutions were scanned in the spectrum mode from 400 nm to 200 nm (fig.1, 2)

Preparation of Calibration Curves

Appropriate dilutions of the standard stock solution were done separately to get 1, 5, 10, 20, 30, 40µg/ml of CEF and LIN, respectively. The absorption spectra of all solutions were recorded between 200-400 nm. The absorbances were measured at 289.0 nm (λmax of CEF), 257.0 nm (λmax of LIN). Beer's lamberts range for CEF and LIN were selected and working calibration curves of both the drugs were plotted separately. (fig.3, 4)

Determination of Absorptivity Value of CEF and LIN

Appropriate dilutions of the standard stock solution were done to get 10 µg/ml of each CEF and LIN, respectively. The absorbances were measured for CEF and LIN at 289nm (λmax of CEF), 257nm (λmax of LIN). The absorptivity values of the drugs were determined at the selected wavelengths. These absorptivity values are the mean of six determinations.

Application of the proposed method for the determination of CEF and AZI in tablets

Twenty tablets were weighed and average weight was calculated. The tablets were crushed to obtain fine powder. Tablet powder equivalent to 10mg of CEF was transferred to 100.0 ml volumetric flask, methanol added, sonicated for 10 minutes and volume was made-up to the mark with methanol. The solution was then filtered through a Whatmann filter paper (No. 41). The filtrate was further diluted with methanol to obtain 6µg/ml of CEF and 18µg/ml of LIN. The concentration of both CEF and AZI were determined by measuring the absorbance of the sample at 289nm, 257nm. Concentration of sample solution was determined by Simultaneous equation method.

Cx =A1 ay2 - A2 ay1/ ax1ay2-ax2ay1.....Eq. (i)

Cy =A1 ax2 - A2 ax1/ ay1ax2-ay2ax1.....Eq. (ii)

Where A1 and A2 are absorbance of the formulation at 257.0 nm and 289.0 nm respectively, ax1 and ax2 are the absorptivity values of CEF at 257 nm and 289 nm respectively. Similarly ay1 and ay2 are the absorptivity value of LIN at 257nm and 289nm respectively. Cx is the concentration of CEF and Cy is the concentration of the LIN. (Table 6)

Validation

The method was validated with respect to linearity, LOD, LOQ, Accuracy and Precision. (Table 2, 3, 4, 5)

RESULTS AND DISCUSSION

The method discussed in the present work provides a convenient and accurate way for simultaneous analysis of CEF and LIN. In simultaneous equation method, wavelengths selected for analysis were 289.0 nm (λmax of Cefixime) and 257.0 nm (λmax of Linezolid). In this method linearity for detector response was observed in the concentration range of 10-40µg/ml for CEF and 10-30µg/ml for LIN. Absorptivities were calculated for both the drugs at selected wavelengths and substituted in equations for determining concentration of CEF and LIN in tablet sample solution. Percent label claim for CEF and LIN in tablet analysis, by this method was found 99.03% for CEF and 98.78%

for LIN. Low values of LOD and LOQ indicated good sensitivity of proposed method. Accuracy of proposed methods was ascertained by recovery studies. The percent recovery for CEF and LIN, by this method, was found 100.51% for CEF & 100.23% for LIN respectively. The proposed method could be employed for routine quality control of Cefixime and Linezolid in combined dose tablet formulation.

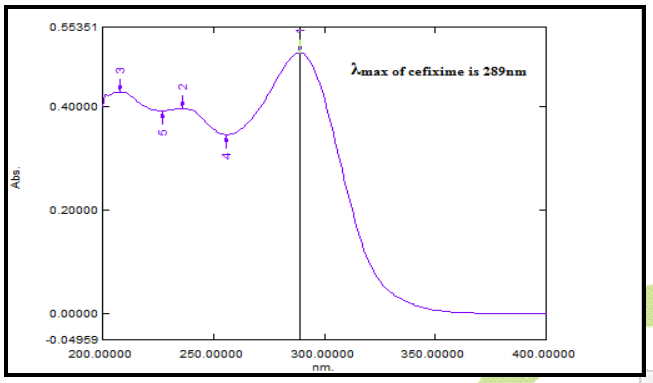

Figure 1: UV-Spectra of Cefixime (10µg/ml)

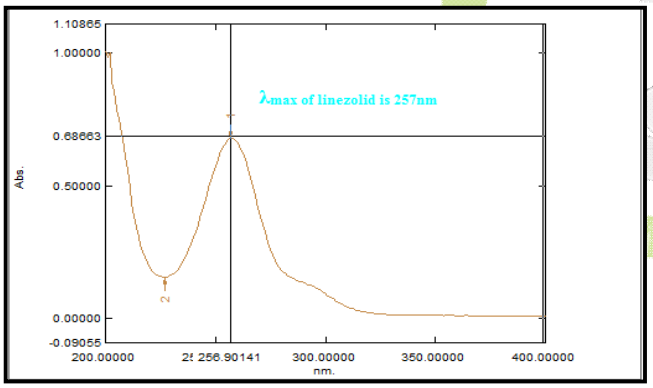

Figure 2: UV-Spectra of Linezolid (10µg/ml)

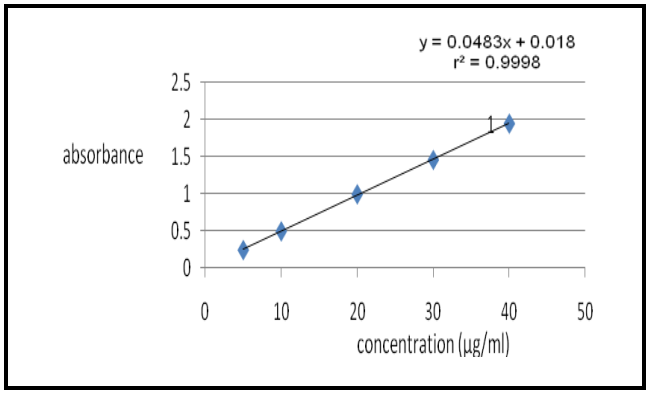

Figure 3: Calibration Curve of Cefixime at 289nm

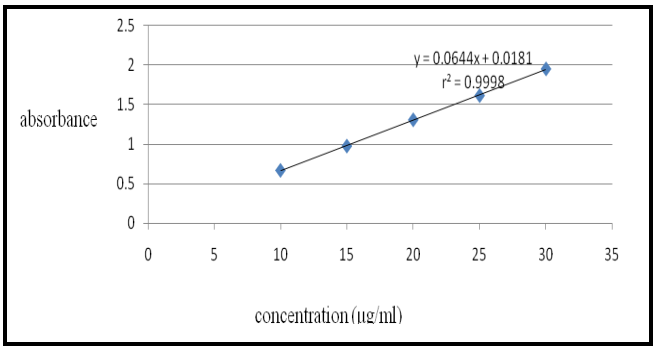

Figure 4: Calibration Curve of Linezolid at 257nm.

Table 1: Summary of Validation Parameters

Sr. No	Parameter	Result	
		Cefixime	**Linezolid**
1	Absorption maxima (λ max)	289nm	257nm
2	Range (µg/ml)	2-50	3-40
3	Linearity (µg/ml)	5-40	10-30
4	Standard regression equation	y = 0.0483x + 0.018	y = 0.0644x + 0.0181
5	Intercept	0.018	0.0181
6	Slope	0.0483	0.0644
7	Correlation coefficient (r^2)	r^2 = 0.9998	r^2 = 0.9998
8	L.O.D (µg/ml)	0.46	0.75
9	L.O.Q (µg/ml)	1.42	2.27
10	Specific absoptivity	Cefixime λ257nm=320	linezolid λ 257nm=650
		Cefixime λ 289nm=500	Cefixime λ 289nm=131

Table 2: Accuracy data of Cefixime

Set	Sample conc.	Standard added	Total conc.	Recovered (µg/ml)	% recovery	Mean % recovery
50	4 µg/ml	2 µg/ml	6 µg/ml	6.10	101.66	99.52
			6 µg/ml	5.91	98.65	
			6 µg/ml	5.88	98.09	
100	4 µg/ml	4 µg/ml	8 µg/ml	8.02	100.25	100.62
			8 µg/ml	8.20	102.5	
			8 µg/ml	7.93	99.11	
150	4 µg/ml	6 µg/ml	10 µg/ml	10.18	101.86	101.39
			10 µg/ml	10.12	101.18	
			10 µg/ml	10.11	101.13	

Table 3: Accuracy data of Linezolid

Set	Sample conc.	Standard added	Total conc.	Recovered (µg/ml)	% recovery	Mean % recovery
50	12 µg/ml	6 µg/ml	18 µg/ml	18.06	100.33	100.13
			18 µg/ml	18.02	100.13	
			18 µg/ml	17.98	99.91	
100	12 µg/ml	12 µg/ml	24 µg/ml	24.00	100.02	100.43
			24 µg/ml	24.10	100.42	
			24 µg/ml	24.20	100.85	
150	12µg/ml	18 µg/ml	30 µg/ml	30.18	100.61	100.15
			30 µg/ml	29.97	99.90	
			30 µg/ml	29.98	99.93	

Table 4: Precision data of Cefixime

Conc. (µg/ml)		Mean absorbance ± SD*		% RSD*	
cefixime	linezolid	257nm	289nm	257nm	289nm
3	9	0.6660 ± 0.0060	0.2660 ±0.0023	0.90	0.88
5	15	1.1176 ± 0.0047	0.4474±0.0049	0.42	1.09
6	18	1.3325 ±0.0049	0.5301±0.0020	0.37	0.38

Table 5: Precision data of Linezolid

Conc. (µg/ml)		Mean absorbance± SD*		% RSD*	
cefixime	linezolid	257nm	289nm	257nm	289nm
3	9	0.6682 ± 0.0065	0.2616 ±0.0039	0.97	1.52
5	15	1.1149 ±0.0066	0.4498 ±0.0029	0.60	0.65
6	18	1.3299 ±0.0039	0.5292±0.0017	0.29	0.34

(where, * is the mean of 3 trials)

Table 6: Result of Assay

Label claim (µg/ml)		Conc. Found (µg/ml)	
Cefixime	Linezolid	Cefixime	Linezolid
6	18	5.97	18.02
6	18	5.83	18.00
6	18	6.02	17.65
6	18	5.93	17.64
6	18	5.96	17.59
Average ± SD*		5.94 ± 0.070	17.78 ± 0.2110
RSD*		1.18	1.19
% Assay found*		99.03	98.78

(where, * is the mean of 5 trials)

CONCLUSION

The developed methods were validated as per ICH guidelines and were found to be within the prescribed limit. It concludes that the developed methods are simple, accurate, sensitive and precise and suitable for both authentic and tablet dosage form.

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