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## Simultaneous Estimation of Cefixime and Linezolid in bulk and tablet dosage form

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### ABSTRACT

Simple area under curve spectrophotometric method for determination of Cefixime and Linezolid in bulk and tablet formulation was developed and validated as per ICH guidelines. The  $\lambda_{max}$  of Cefixime and Linezolid were found to be 289nm and 257nm respectively. The linearity range was found 2-12 $\mu$ g/ mL for Cefixime and 5-30 $\mu$ g/mL for Linezolid. In the tablets dosage form Cefixime and Linezolid were estimated as 99.92% and 99.94% respectively. The lower limit of detection (LOD) for Cefixime and Linezolid was found to be 0.039 $\mu$ g/ml and 1.11 $\mu$ g/ml respectively. and the limit of quantization (LOQ) was determined as the lowest concentration for Cefixime and Linezolid was found to be 0.118 $\mu$ g/mL and 3.37 $\mu$ g/ml respectively. The validated spectrophotometric method employed proved to be simple, economical, precise and accurate.

**Keywords:** Cefixime, Linezolid, UV Spectroscopy, AUC Method.1.

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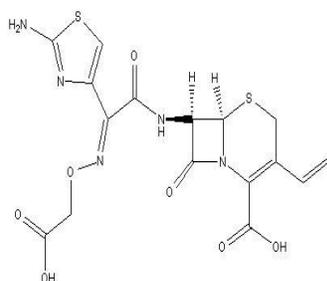
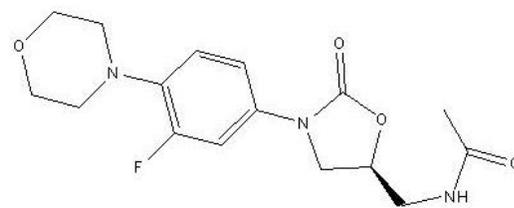
## INTRODUCTION

Cefixime chemically it is (6R,7R)-7-[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-[(carboxymethoxy)imino]acetamido]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid trihydrate. It is a third generation cephalosporin anti-bacterial drug given by oral route in the treatment of susceptible infections including gonorrhea, otitis media, pharyngitis and urinary tract infection. Cefixime binds to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall, causing the inhibition of the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that cefixime interferes with an autolysin inhibitor.<sup>1</sup>

Linezolid chemically it is *N*-{[(5S)-3-[3-fluoro-4-(morpholin-4-yl) phenyl]-2-oxo-1,3-oxazolidin-5-yl]methyl}acetamide is first drug of the oxazolidinone class of antibiotic drug and it is used as antibacterial agent. Linezolid selectively inhibits bacterial protein synthesis through binding to sites on the bacterial ribosome and prevents the formation of a functional 70S-initiation complex. Specifically, linezolid binds to a site on the bacterial 23S ribosomal RNA of the 50S subunit and prevents the formation of a functional 70S initiation complex, which is an essential component of the bacterial translation process. The results of time-kill studies have shown linezolid to be bacteriostatic against enterococci and staphylococci.<sup>2</sup>

Various UV Spectrophotometric methods are reported in literature for the analysis of Cefixime and Linezolid in single and in combination with other drugs. Mehul Patel *et al* worked on the development and validation of first order derivative spectrophotometric method for simultaneous estimation of cefixime trihydrate and moxifloxacin hydrochloride in combined dosage form. The solvent used was methanol and determinations were made at 295.8 nm for cefixime trihydrate and 288.8 nm for moxifloxacin hydrochloride.<sup>3</sup> NarendraNyola *et al* worked on simultaneous estimation of Azithromycin and cefixime in API's and Pharmaceutical dosage form by spectrophotometry. The solvent used was methanol, the maximum wavelength of Azithromycin and Cefixime were found to be 235 nm and 288 nm respectively.<sup>4</sup> Santhi N. *et al* worked on simultaneous estimation of cefixime and ofloxacin in bulk and tablet dosage form. The solvent used was ethanol, Method A involves simultaneous equations at 290.4 nm and 297.4 the wavelength maxima of cefixime and ofloxacin respectively. Method B involves Absorbance ratio method at 282.0 nm(iso-absorptive point) and 297.4 nm wavelength maxima of ofloxacin.<sup>5</sup> Prashanti P. *et al* worked on development and validation of UV Spectrophotometric method for estimation of Linezolid in bulk and pharmaceutical formulation. The solvent used

was methanol, the wavelength maxima were found to be 251 nm.<sup>6</sup> Vanitha P. *et al* worked on method development and validation of Linezolid in bulk and formulation using UV Spectrophotometric method. The solvent used was 20% Acetonitrile, the wavelength maxima were found to be 253 nm.<sup>7</sup>

**Cefixime****Linezolid****Figure 1: Chemical Structure of Cefixime and Linezolid**

## MATERIALS AND METHODS

### Apparatus

Shimadzu UV-1800 double beam spectrophotometer connected to a computer loaded with Shimadzu UV Probe software was used for all the spectrophotometric measurements. The absorbance spectra of the reference and test solutions were carried out in 1cm quartz cells over the range of 200-400 nm. The instrument has the scan speed of 600 nm min<sup>-1</sup>. All weighing were done on electronic balance (Model Shimadzu AUV-220D).

### Reagents and Chemicals

Cefixime and Linezolid were obtained as gift sample from Wockhardt Limited, Aurangabad, India and Mylan Pharmaceuticals, Hyderabad, India respectively. Zifi-Turbo a Combination tablet of Cefixime (400 mg) and Linezolid (600mg) was obtained as gift sample from FDC Ltd. Aurangabad, India. Methanol AR grade were purchased from Fischer Limited, Mumbai, India.

### Preparation of standard solution

10 mg each of Cefixime and Linezolid were weighed separately and transferred in two different 100ml volumetric flasks. Both the drugs were dissolved in 25 ml of methanol solvent by vigorous shaking and then volume was made up to mark with methanol to obtained final concentration of 100 µg/ml of each component. For each drug, appropriate liquid were pipette out from the standard stock solution into a series of 10mL volumetric flasks, to get a set of dilutions for each drug.

### Selection of Analytical wavelength

By appropriate dilutions with methanol 6 µg/ml solution of Cefixime and 15 µg/ml solution of

Linezolid were prepared separately. These were scanned in the spectrum mode from 400 nm-200 nm. As shown in figure the wavelength range for Cefixime was chosen between 270-300 nm and for Linezolid it was chosen between 234.5-280 nm.

### Determination of 'X' values

$X = \text{AUC of component between selected wavelength range} / \text{Concentration of that component in gm/lit.}$

### Procedure for analysis of tablet formulation

Twenty tablets of Cefixime and Linezolid in combination were weighed; their average weight was determined and finally crushed to powder sample. From the triturate, tablet powder equivalent to 40 mg of Cefixime and 60 mg of Linezolid was weighed and transferred to 100 ml volumetric flask and dissolved in 50 ml methanol and Finally the volume was made upto the mark with methanol. The solution is subjected to ultra sonification for 30 min. and then filtered through Whatman filter paper No.41. This tablet solution was further diluted to obtain 40 µg/ml of Cefixime and 60 µg/ml of Linezolid respectively. The mixed sample solutions were analyzed to obtain spectra and the AUC is recorded using wavelength range from 270–300nm for Cefixime and 234.5–280nm for Linezolid were noted.

### Calibration curve

#### Method: Area under Curve Method

For the selection of analytical wavelength, 6 µg/mL solution of Cefixime and 15 µg/mL solution of Linezolid was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. From the spectra of drugs  $\lambda$  max of Cefixime and Linezolid, was selected 289nm and 257nm respectively for the analysis. The calibration curve was prepared in the concentration range of 2-12µg/mL at 289nm for Cefixime and 5-30µg/mL at 257nm for Linezolid. By using the calibration curve, different concentrations of the sample solution were calculated.

#### Equation used for determination of concentrations of Cefixime and Linezolid:

$$C_{\text{Cefixime}} = \frac{X^{\text{Linezolid}}_{270-300} \times \text{AUC}_{234.5-280} - X^{\text{Linezolid}}_{234.5-280} \times \text{AUC}_{270-300}}{X^{\text{Linezolid}}_{270-300} \times X^{\text{Cefixime}}_{234.5-280} - X^{\text{Linezolid}}_{234.5-280} \times X^{\text{Cefixime}}_{270-300}}$$

$$C_{\text{Linezolid}} = \frac{X^{\text{Cefixime}}_{234.5-280} \times \text{AUC}_{270-300} - X^{\text{Cefixime}}_{270-300} \times \text{AUC}_{234.5-280}}{X^{\text{Linezolid}}_{270-300} \times X^{\text{Cefixime}}_{234.5-280} - X^{\text{Linezolid}}_{234.5-280} \times X^{\text{Cefixime}}_{270-300}}$$

Where  $\text{AUC}_{234.5-280}$  and  $\text{AUC}_{270-300}$  are the area under curves of solution at wavelength range between 234.5-280 nm (Linezolid) and 270-300 nm (Cefixime) respectively.

## RESULTS AND DISCUSSION

Calibration standards for Cefixime covering the range of 2-12 µg/ml and for Linezolid the range of 5-30 µg/ml were prepared by the method mentioned above and the serial dilutions were made with methanol. The spectrum was presented in Figure 2. The calibration curve was obtained by plotting the intensity by absorbance of the Cefixime and Linezolid versus respective analyte concentration. Regression analysis of the calibration curve showed a linear relationship between the intensity of absorbance of Cefixime and Linezolid respectively. The wavelength range for Cefixime was chosen between 270-300 nm and for Linezolid it was chosen between 234.5-280 nm. The various validation parameters are presented in Table 9.

**Table 1: Calculation of 'X' Values for Cefixime and Linezolid**

Components	X Value at	
	270 - 300 nm	234.5 – 280 nm
Cefixime	9.934	6.35
Linezolid	26.78	38.206

**Table 2: Calibration Curve Data for Cefixime by UV**

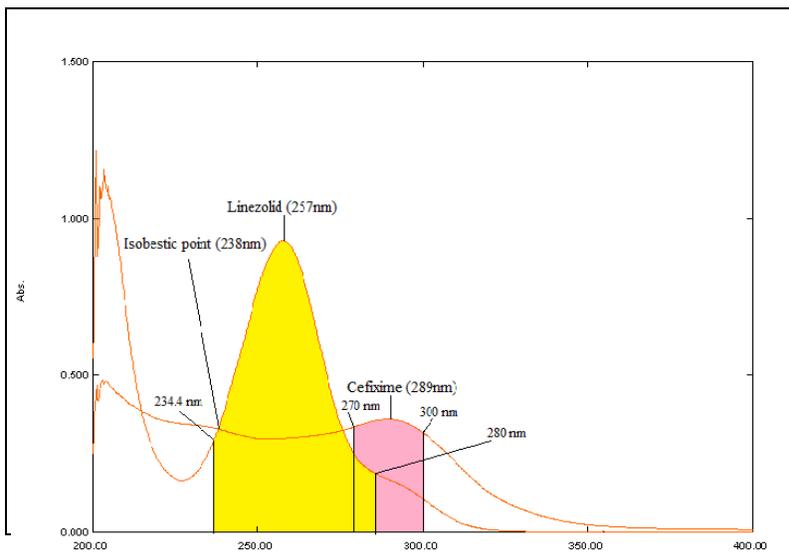
Conc. in µg/ml	Absorbance
2	0.109
4	0.198
6	0.301
8	0.399
10	0.505
12	0.612

**Table 3: Calibration Curve Data for Linezolid by UV**

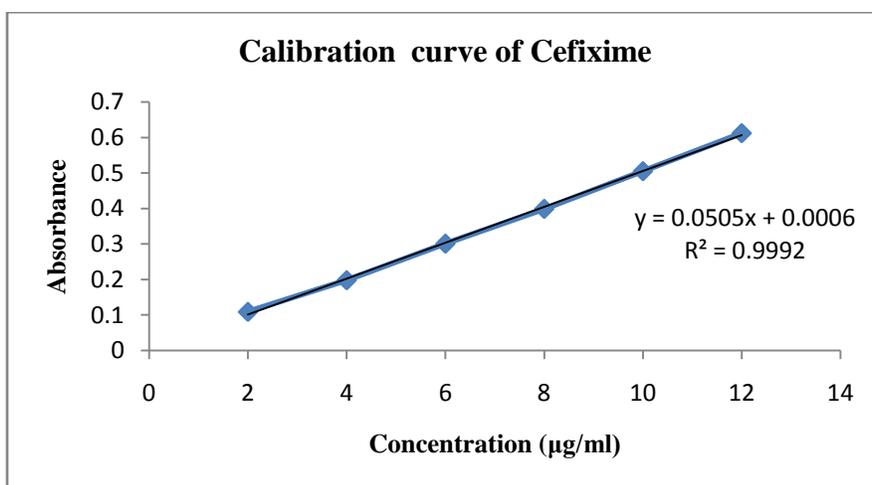
Conc. in µg/ml	Absorbance
5	0.321
10	0.652
15	0.942
20	1.261
25	1.543
30	1.881

**Table 4: Data for Bulk Drug Analysis**

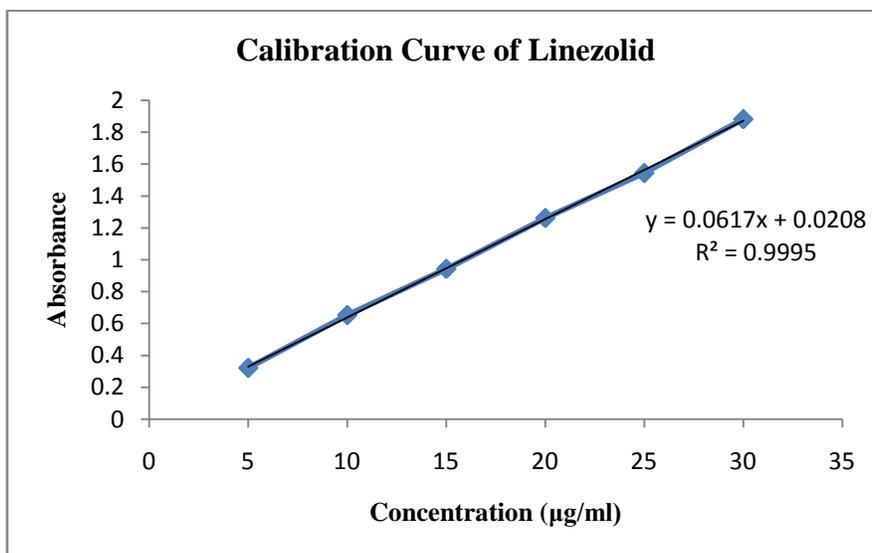
Sr. No.	Amount present in µg/ml		Amount found in µg/ml		Amount found in %	
	Cefixime	Linezolid	Cefixime	Linezolid	Cefixime	Linezolid
1	20	30	19.87	29.76	99.35	99.2
2	20	30	19.90	29.79	99.5	99.3
3	20	30	19.84	29.91	99.2	99.7
4	20	30	19.86	29.78	99.3	99.26
5	20	30	19.89	29.86	99.45	99.53
% Mean					99.36	99.39



**Figure 2: Overlay Spectra Showing Area under Curve of Cefixime and Linezolid**



**Figure 3: Calibration curve of Cefixime by UV**



**Figure 4: Calibration curve of Linezolid by UV**

**Table 5: Data for Tablet Analysis**

Sr. No.	Label Claim mg/tab		Amount found in mg/tab		% Label Claim	
	Cefixime	Linezolid	Cefixime	Linezolid	Cefixime	Linezolid
1	400	600	399.70	600.20	99.92	100.03
2	400	600	400.10	599.80	100.025	99.96
3	400	600	399.80	600.10	99.95	100.016
4	400	600	400.09	599.70	100.022	99.95
5	400	600	400.11	599.90	100.027	99.98
6	400	600	400.08	600.12	100.02	100.02
% Mean					99.99	99.99

**Validation according to ICH guidelines****Linearity**

For quantitative analysis of Cefixime and Linezolid, the calibration curves were plotted for each concentration ranges. The linearity ranges for zero order derivative, first order derivative and second order derivative was found to be for Cefixime 2-12 µg/ml and for Linezolid found to be 5-30 µg/ml respectively.

**Limit of detection (LOD) and Limit of Quantitation (LOQ)**

The LOD and LOQ of Cefixime and Linezolid by the proposed methods were determined using calibration standards. LOD and LOQ values were calculated as  $3.3 \sigma/S$  and  $10 \sigma/S$ , respectively, where S is the slope of the calibration curve and  $\sigma$  is the standard deviation of y-intercept.

**Accuracy**

The accuracy was determined by standard addition method. Three different levels (80%, 100% and 120%) of standards were spiked to commercial tablets in triplicate. The mean of percentage recoveries and the %RSD was calculated.

**Table 6: Data for Recovery Studies by Area under Curve Method**

	Cefixime			Linezolid		
	Level of % Recovery			Level of % Recovery		
	80	100	120	80	100	120
Amount Present (mg)	400	400	400	600	600	600
Amount of Standard Added (mg)	320	400	480	480	600	720
Total Amount Recover (mg)	719.8	799.1	879.3	1079.5	1198.9	1319.7
% Recovery	99.97	99.88	99.92	99.95	99.90	99.97
% Mean	99.92			99.94		
SD	0.06164			0.02943		
% RSD	0.0616			0.02944		

**Specificity**

Comparison of the zero-, first- and second- order derivative spectrum of Cefixime and Linezolid in bulk drug with drug formulation (Zifi-Turbo) solutions shows that the wavelengths of

maximum and minimum absorbance do not change.

### Precision

The reproducibility of proposed method was determined by performing tablet assay at different time intervals (3 hour interval) on same day (Intra-day precision) and on three different days (Inter-day precision) for Cefixime and Linezolid at the concentration 2-6 µg/ml, and 5-25 µg/ml respectively.

**Table 7: Intraday and Interday Precision of Cefixime**

Replicate	Intra Day			Inter Day		
	4(µg/ml)	6(µg/ml)	8(µg/ml)	4(µg/ml)	6(µg/ml)	8(µg/ml)
1	0.198	0.301	0.399	0.198	0.301	0.399
2	0.197	0.299	0.397	0.191	0.295	0.393
3	0.195	0.298	0.395	0.189	0.292	0.389
Mean	0.196	0.299	0.397	0.192	0.296	0.393
SD	0.00124	0.00124	0.00163	0.0038	0.00374	0.00411
%RSD	0.63	0.414	0.410	1.979	1.263	1.04
Mean of % RSD	0.484			1.42		

**Table 8: Intraday and Interday Precision of Linezolid**

Replicate	Intra Day			Inter Day		
	5(µg/ml)	10(µg/ml)	15(µg/ml)	5(µg/ml)	10(µg/ml)	15(µg/ml)
	Absorbance					
1	0.652	0.942	1.261	0.652	0.942	1.261
2	0.651	0.940	1.259	0.645	0.935	1.255
3	0.649	0.938	1.258	0.641	0.931	1.250
Mean	0.650	0.94	1.259	0.646	0.936	1.255
SD	0.00124	0.00163	0.00124	0.0045	0.0045	0.0044
%RSD	0.190	0.173	0.0984	0.696	0.480	0.350
Mean of% RSD	0.153			0.508		

**Table 9: Summary of Validation Parameters**

Parameter	Data for Cefixime	Data for Linezolid
Linearity range	2-12 µg/ml	5-30 µg/ml
Regression equation	$y = 0.0505x + 0.0006$	$y = 0.0617x + 0.0208$
Correlations coefficient	0.9992	0.9995
Limit of Detection (µg/mL)	0.039	1.11
Limit of Quantitation (µg/mL)	0.118	3.37
Accuracy	99.92%	99.94%
Specificity	Specific (Spectra match with standard drug)	Specific (Spectra match with standard drug)
Robustness	Robust	Robust
Ruguddeness	%RSD Less than 1%	%RSD Less than 1%
Precision (RSD, %)		
Intraday (n=3)	0.484	0.153
Interday (n=3)	1.42	0.508

## CONCLUSION

From above it is concluded that the simple, economical, precise and accurate spectrophotometric Area under curve method for determination of Cefixime and Linezolid in bulk and tablet formulation was developed and validated as per ICH guidelines. Thus it can be used as IPQC test and for routine simultaneous determination of Cefixime and Linezolid in tablet dosage form.

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