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Method Development and Validation for Simultaneous Estimation of cefixime And Linezolid In Bulk and Pharmaceutical Dosage Forms

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ABSTRACT

A simple, rapid, sensitive reverse-phase high-performance liquid chromatography method was developed and validated for simultaneous estimation of Cefixime and linezolid ,at single wavelength of 263nm.chromotographic separation was performed on an enable aligent zorabax(thermo) column(250nmx4.6mm ID particle size 5 um) and a mobile phase consisting of acetonitrile and water(70:30v/v) at a flow rate of 1.0ml/min. the calibration curve was linear($r^2 \geq 0.0999$) over the concentration range. 400-1200µg/mL of cefixime and 1200-3600µg/mL of linezolid. the limit of quantification was 9.709µg/ml for Cefixime and 9.482µg/ml for linezolid no interference was found by the excipients in the synthetic mixture. The proposed methods were validated for international conference on harmonization guidelines for linearity, accuracy, precision, and robustness for estimation of Cefixime and linezolid in bulk and synthetic mixture, and the results were found to be satisfactory

Keywords: cifexime, linezolid, RP-HPLC Validation

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INTRODUCTION

Cefixime[1,2] Figure 1 an antibiotic, is a third-generation cephalosporin like ceftriaxone and cefotaxime[1]. Chemically, it is known as (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. 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.

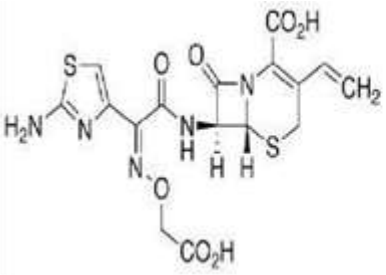


Figure 1: Structure ofcefixime

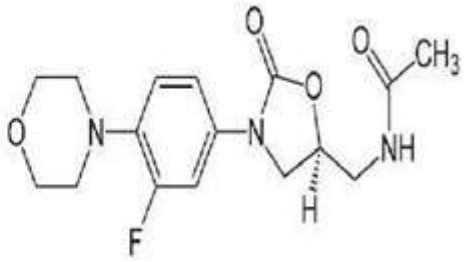


Figure 2: Structure of Linezolid

Linezolid[3] Figure 2 is a synthetic antibiotic, the first of the oxazolidinone class. Chemically, it is known as N-{[(5S)-3-[3-fluoro-4-(morpholin-4-yl) phenyl [-2-oxo-1,3-oxazolidin-5-yl]methyl}acetamide.mIt selectively inhibits bacterial protein synthesis through binding to sites on the bacterial ribosome and prevents the formation of a functional 70S-initiation complex. A combined dosage form of the above drugs in the brand name **LCZ-2**, (Label claim: cefixime 200mg, linezolid 600mg) available in local pharmacy is used to treat different types of bacterial infections, such pneumonia, skin infections, and infections that are resistant to other antibiotics

The literature survey revealed that there are only two HPLC methods[4,5] developed for the estimation of cefixime and linezolid in combination dosage forms. The present study was aimed to develop a new stability indicating method for simultaneous estimation of cefixime and linezolid in combined pharmaceutical dosage forms.

MATERIALS AND METHOD

Reagents & Materials:

HPLC-grade Acetonitrile were purchased from E. Merck (Mumbai, India). HPLC grade water (merck), purchased from E. Merck was used to prepare all solutions. Cefixime and Linezolid pure powder was kindly gifted by iifa Health care Pvt Ltd, New Delhi, India and their dosage form(Tablets) trade name LCZ-2, (Label claim: cefixime 200mg, linezolid 600mg) was procured from the local pharmacy

Instrumentation:

The HPLC system consisted of Intelligent HPLC pump model (Jasco PU2080 Plus) with sampler

programmed at 10 µL capacity per injection was used. The detector consisted of a UV/ VIS (Jasco UV 2075 Plus). Data was integrated using Empower 2software, integrated to Dell computer system. The column used was, Aligent, Zorbax- C18 (4.6 mm I.D. × 250mm L).

Mobile Phase Preparation:

The mobile phase selected was acetonitrile: water in the ratio of 70:30(v/v), and before analysis mobile phase was degassed.

Diluent Preparation:

Mobile phase was used as a diluent in the present assay.

Standard Preparation:

The standard stock solution 1.0mg/mL of Cefixime and Linezolid were prepared separately by dissolving 50 mg of each drug in 50mL mixture of acetonitrile and water (70:30 v/v). From the standard stock solution, mixed standard solution was prepared to contain 400-1200µg/mL of cefixime and 1200-3600µg/mL of linezolid.

Sample Preparation:

To determine the content of cefixime and linezolid in conventional tablets (Brand name: LCZ-2, Label claim: cefixime 200mg, linezolid 600mg), twenty tablets were weighed, their mean weight determined and finely powdered. The average weight of the tablet triturate equivalent to 500 mg of cefixime and 100mg of linezolid was transferred into a 100mL volumetric flask containing 60-65mL methanol, sonicated for 30 min and diluted to 100mL with methanol. The resulting solution was centrifuged at 3000rpm for 5 min and the drug content of the supernatant was extracted (5.0mg/mL and 1.0mg/ml for cefixime and linezolid respectively). Then 1.0 mL of the above filtered solution was diluted to produce a concentration of 400-1200µg/mL of cefixime and 1200-3600µg/mL of linezolid and the solution was filtered through 0.45 mm nylon filter before injecting into HPLC system. 10 µL volume of this sample solution was injected into HPLC system, six times, under the conditions

Chromatographic Conditions:

Mobile phase consisted of a mixture of Acetonitrile: Water (70: 30, v/v), at a flow rate of 1.0 mL/min with detection at 263nm. The mobile phase was filtered through a 0.2 micron membrane filter and degassed. The injection volume was 10µL and analysis was performed at ambient temperature.

RESULTS AND DISCUSSION

Development and optimization of the HPLC method:

Several tests were performed in order to get satisfactory separation-resolution of cefixime and linezolid in mobile phases with various ratios of organic phase and water by using C_{18} column. Acetonitrile with water in the ratio (70:30) was preferred because it resulted in a greater response to cefixime and linezolid. The retention of cefixime and linezolid on analytical column was evaluated at 2.553 and 3.624 min at a flow rate of 1.0 $mL.min^{-1}$. The injection volume was 10µl. The retention time of standard and sample for were satisfactory with good resolution. Under these conditions, the analyte peak was well-defined and free from tailing was obtained.

METHOD VALIDATION:

System Suitability:

A system suitability test of the chromatographic system was performed before each validation run. Five replicate injections of standard preparation were injected and asymmetry, theoretical plates, resolution and % RSD of peak area were determined for same. Acceptance criteria for system suitability, Asymmetry not more than 2.0, theoreticals not less than 2000 for cefixime and 5000 for linezolid and the % RSD of peak area not more then 2.0, were full fill during all validation parameter

Blank and **Placebo Interference:**

A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of blank solution Figure 3a showed no peaks at the retention time of cefixime and linezolid peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of cefixime and linezolid in tablets. Similarly chromatogram of placebo solution Figure 3b showed no peaks at the retention time of cefixime and linezolid peaks. This indicates that the placebo used in sample preparation do not interfere in estimation of cefixime and linezolid in their formulations.

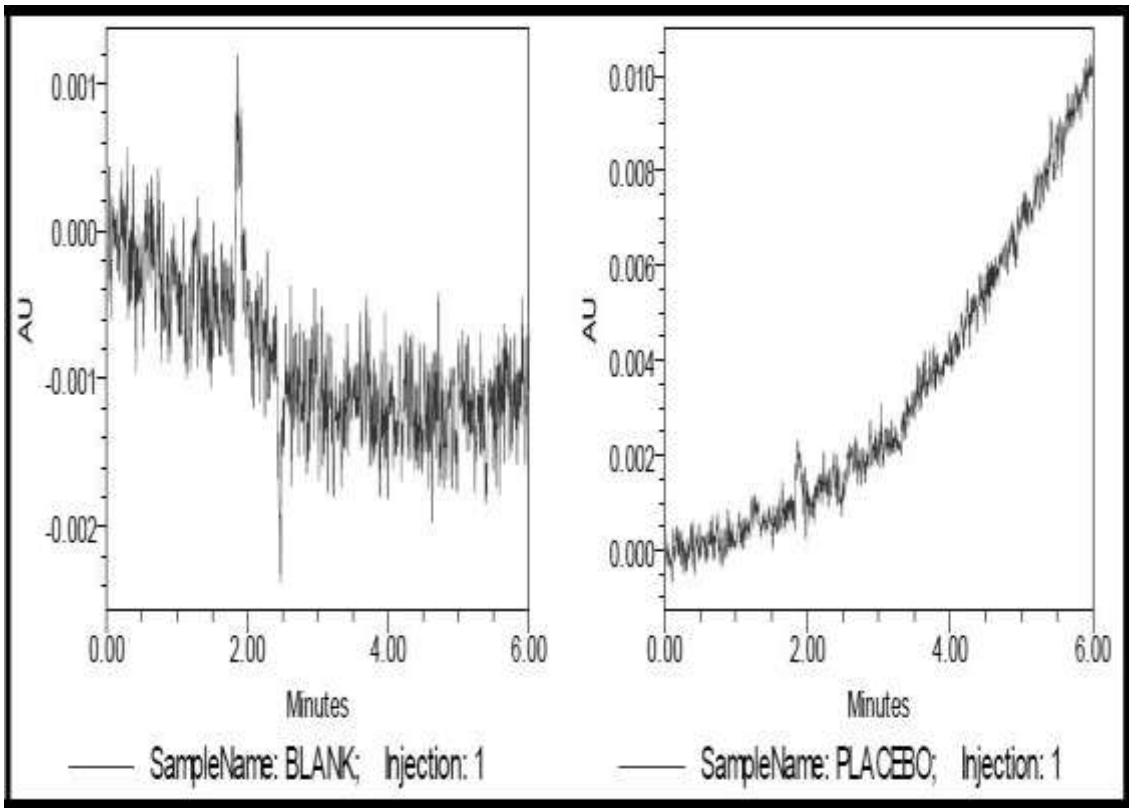
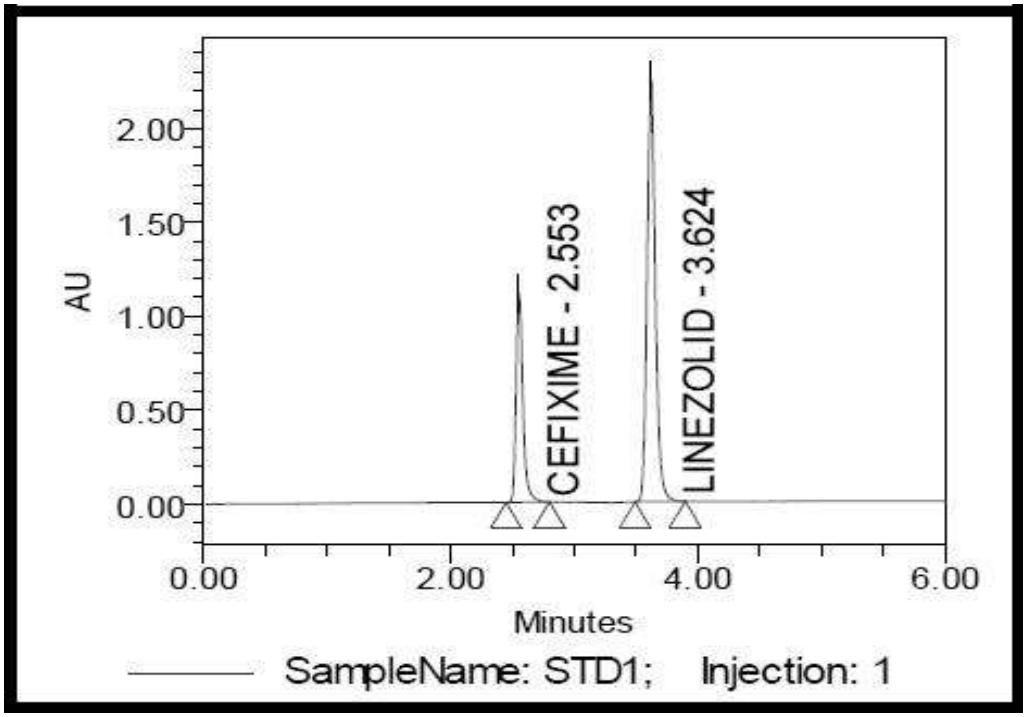


Figure 3a & 3b Typical HPLC Chromatogram Showing the no Interference of Blank and Placebo for Cefixime and Linezolid



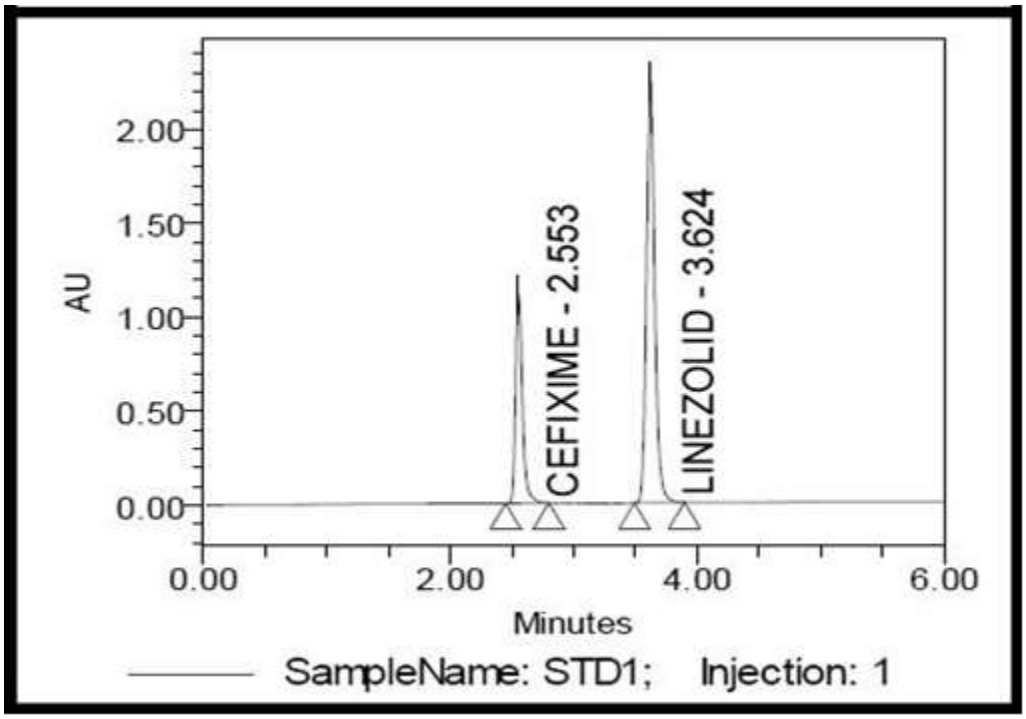


Figure 4 Typical HPLC Chromatogram cefixime And Linezolid

Linearity:

The standard curve was obtained in the concentration range of 400-1200µg/mL for cefixime and 1200-3600µg/mL for linezolid. Evaluation of two drugs were performed with PDA detector at 263nm, peak area recorded for all the peaks and are given in the Table 1a & b. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r^2] of standard curve were plotted and calculated and are given in Figure 5a & Table 1a for cefixime and Figure 6b & Table 2b for linezolid respectively. The slope and intercept value for calibration curve was y = 41850x + 22620 (R2 = 0.999) for cefixime and y = 10277x - 2437 (r^2 = 1.000) for linezolid. These results showed that an excellent correlation exists between peak area and concentration of drugs within the concentration range indicated above.. The LOD value for cefixime and linezolid were found to be 2.913µg/mL and

LINEARITY STUDY FOR CEFIXIME		
% LEVEL (APPROX.)	CONC. µg/ml	AREA
50	400	2114445
75	600.00	3169573
100	800.00	4229256
125	1000	5188456
150	1200	6336214
Slope		41850
Intercept		22620
RSQ(r2)		0.999
LOD		2.913
LOQ		9.709

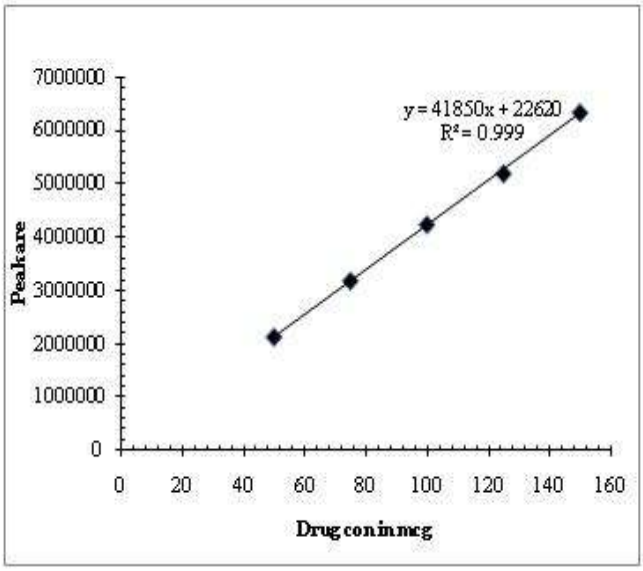


Table :1a & Figure 5a: **Linearity Studies & Curve Of Cefixime by the Proposed Method**

LINEARITY STUDY FOR LINEZOLID		
% LEVEL (APPROX.)	CONC. µg/mL	AREA
50	1200	5137352
75	1800	7706992
100	2400	10291111
125	3000	12802750
150	3600.00	15436095
Slope		10277
Intercept		-2437.0
RSQ(r2)		1.00
LLD (µg/ml)		2.844
LLQ (µg/ml)		9.482

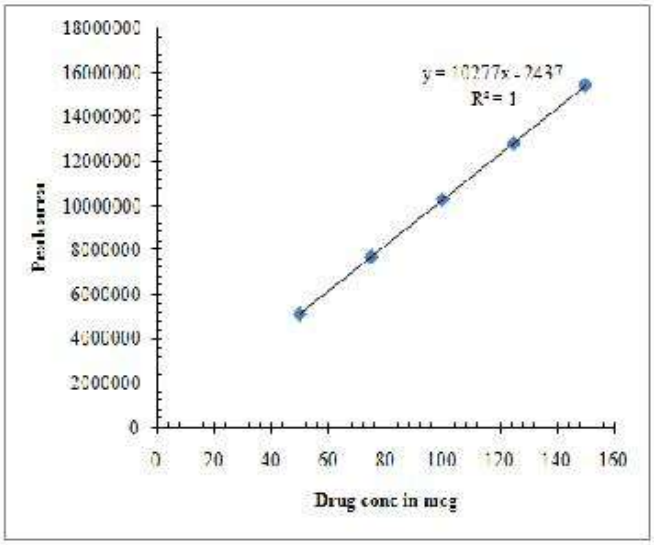


Table: 1b & Figure:5b Linearity Studies & Curve of Linezolid by the Proposed Method

Precision:

The precision of the method was demonstrated by interday and intraday variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the interday variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks and percentage RSD were calculated Table 2 The method precision study for six sample preparations in showed %RSD of 0.041 for cefixime and 0.11 for linezolid respectively. From the data obtained, the developed RP-HPLC method was found to be

precise.

Table 2: Method Precision Studies For Cefixime and Linezolid By The Proposed Method

For cefixime		**For linezolid**
Set-1	6339376	15497998
Set-2	6335703	15477319
Set-3	6339203	15493713
Set-4	6336282	15475259
Set-5	6332419	15483088
Set-6	6337893	15447911
Over All Avg.	6336813	15479215
Over All Std Dev.	2617.967	17760.51
Over All %RSD	0.041	0.011

Accuracy:

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on composite blend collected from 20 tablets of cefixime and linezolid, analyzed as per the proposed method. The percentage recoveries with found in the range of 100 for cefixime and for linezolid. From the data reported in Table 3a&b , reported that the developed RP-HPLC method was found to be accurate for cefixime and linezolid assay.

TABLE: 3a: **Recovery Studies For Cefixime And Linezolid By The Proposed Method**

CEFIXIME

Spiked Level	**Sample Weight**	**Sample Area**	**µg/mL added**	**µg/mL found**	**% Recovery**	**% Mean**
						100
50%	533.38	2117320	396.404	397.26	100	
50%	533.38	2119871	396.404	397.74	100	
50%	533.38	2114783	396.404	396.79	100	
50%	533.38	2118526	396.404	397.49	100	
						100
50%	533.38	2112747	396.404	396.40	100	
50%	533.38	2117286	396.404	397.26	100	
100%	1066.75	4220180	792.800	791.81	100	
100%	1066.75	4225678	792.800	792.84	100	
100%	1066.75	4226376	792.800	792.97	100	
150%	1600.10	6339376	1189.181	1189.42	100	
150%	1600.10	6335703	1189.181	1188.73	100	
150%	1600.10	6339203	1189.181	1189.39	100	100
150%	1600.10	6336282	1189.181	1188.84	100	
150%	1600.10	6332419	1189.181	1188.12	100	
150%	1600.10	6337893	1189.181	1189.14	100	

Table: 3.b: **Recovery Studies For Cefixime And Linezolid By The Proposed Method**

LINEZOLID

Spiked Level	Sample Weight	Sample Area	µg/mL added	µg/mL found	% Recovery	% Mean
50%	445.65	5139188	1196.411	1197.40	100	100
50%	445.65	5137658	1196.411	1197.04	100	
50%	445.65	5138905	1196.411	1197.33	100	
50%	445.65	5130918	1196.411	1195.47	100	
50%	445.65	5133534	1196.411	1196.08	100	100
50%	445.65	5134330	1196.411	1196.27	100	
100%	891.30	10245603	2392.800	2387.16	100	
100%	891.30	10219054	2392.800	2380.98	100	
100%	891.30	10269518	2392.800	2392.73	100	
150%	1337.00	15497998	3589.144	3610.94	101	
150%	1337.00	15477319	3589.144	3606.12	100	
150%	1337.00	15493713	3589.144	3609.94	101	100
150%	1337.00	15475259	3589.144	3605.64	100	
150%	1337.00	15483088	3589.144	3607.46	101	
150%	1337.00	15447911	3589.144	3599.27	100	

Robustness:

The robustness study of the developed assay method for cefixime and linezolid were established in all variance conditions. Assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence, the analytical method would be concluded as robust (Table 4).

Table.4: Robustness Studies of the Proposed RP-HPLC Method

Robust Conditions		Cefixime RT	Cefixime Peak Area	Linezolid RT	Linezolid Peak Area
Flow Rate	0.8 mL/min	2.567	6392142	14	3.72116483088
	1.2 mL/min	2.662	6362822	82	3.87515997911
Temp	30 ^{o}C	2.661	6532419		3.84616163088
	40 ^{o}C	2.862	6637993		3.97916654321

Assay in formulations:

The robustness study of the developed assay method for cefixime and linezolid were established in all variance conditions. Assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence, the analytical method would be concluded as robust.

Table 5: Analysis of Marketed Tablets by the Proposed RP-HPLC Method

Drug	Label claim	Quantity found*	%RSD	%Assay
Cefixime	200mg	199.92	0.0213	99.96
Linezolid	600mg	599.98	0.075	99.99

*Average of six determinations

CONCLUSION

A simple, rapid, sensitive and economical RP-HPLC method has been developed for the estimation of cefixime and linezolid in pure and also in combined dosage forms. The credibility of the proposed method has been established by validation as per the ICH guidelines. In this proposed method the linearity is observed in the concentration range of 400-1200µg/mL and 1200-3600µg/mL with co-efficient of correlation, (r2) = 0.9991 and (r2) = 1.0 for cefixime and linezolid, respectively at 263nm. The results of the analysis of pharmaceutical formulation by the proposed method are highly reproducible and reliable and it is in good agreement with the label claim of the drug. The simplicity, selectivity and robustness achieved by the proposed method advocated that **"**the proposed RP-HPLC method can be used for the routine analysis of the Cefixime and linezolid in combined dosage form without any interference of excipients

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