Development and Validation of Analytical Method for Simultaneous Estimation of Cefuroxime Axetil and Linezolid in Tablet Dosage form

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Abstract:

A simple, precise, accurate, sensitive and rapid Simultaneous Equation method was developed for simultaneous estimation of Cefuroxime Axetil(CEF) and Linezolid(LIN) in Tablet dosage form. The proposed method was applied for the determination of Cefuroxime Axetil and Linezolid in Tablet formulation, for determination of sampling using two wavelengths, CEF and LIN were scanned in 200-400 nm range and sampling wavelengths were 276.60 nm for CEF and 257.40 nm for LIN are selected for development and validation of simultaneous equation method. For this method linearity observed in the range of 2-6 µg/ml for CEF and 2.4-7.2 µ g/ml for LIN and in their pharmaceutical formulation with mean percentage recoveries 99.90± 0.005 and 100.02± 0.009, respectively. The method was validated according to ICH guidelines and can be applied for routine quality control testing.

Keywords: Spectroscopic method, simultaneous equation method, Cefuroxime Axetil and Linezoli

1. Introduction

The aim of the present work was to develop a new simple, precise, accurate and rapid method for the simultaneous determination of components having overlapping spectra in tablet formation. To prove the ability of the newly described method in resolving the overlapping spectral data and simultaneous determination of each component, it was applied for the analysis of a mixture of Cefuroxime Axetil (CEF) and Linezolid (LIN) formulated together in the form of tablet widely used for the treatment of bacterial infection. [1-5]

Cefuroxime Axetil is chemically (1 RS)-1-(acetyloxy)ethyl(6R,7R)-3-[(carbamoyloxy)methyl]-7[[(Z)-2-(furan-2-yl)-2-(methoxyimino)acethyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate. Cefuroxime Axetil is a bactericidal in action. Like other cephalosporins, the antibacterial activity of the drug results from inhibition of mucopeptide synthesis in the bacterial cell wall. That is used for treating bacterial infection. Linezolid is chemically N-[(5S)-3-[3-fluoro-4-(4-morpholinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl]acetamide. Linezolid is a member of a new structural class of antibiotics, oxazolidinones. The oxazolidinones have a good activity against Gram-positive bacteria. They act uniquely by inhibiting the formulation of protein synthesis initiation in Gram-positive bacteria. Combination of Cefuroxime Axetil and Linezolid is used to treat bacterial infection. [6-16]

(A) (B)

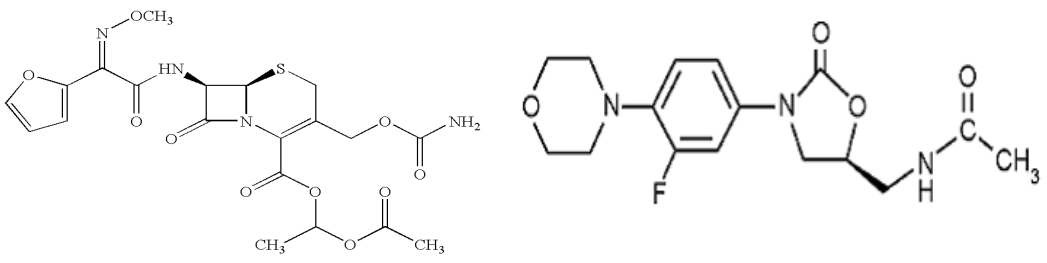

Fig. 1 **(A)** is Structure of Cefuroxime Axetil and **(B)** is structure of Linezolid

1.1. Theory

We can find out concentration of both the drug from combination mixture using the simultaneous equation method. In this method using the absorbance of both the drug and mixture at their wavelength and put this value in following equation and we can find out the concentration of drugs present in combination.

$$Cx = \frac{(A_2 \times ay_1) - (A_1 \times ay_2)}{(ay_1 \times ax_2) - (ay_2 \times ax_1)} \quad \textbf{(1)}$$

$$Cy = \frac{(A_1 \times ax_2) - (A_2 \times ax_1)}{(ay_1 \times ax_2) - (ay_2 \times ax_1)} \quad \textbf{(2)}$$

Where,

Cx = Concentration of drug X

Cy = Concentration of drug Y

ax1 = Absorptivity of drug A at wavelength 1

ax2 = Absorptivity of drug A at wavelength 2

A1 = Absorbance of mixture at wavelength 1

A2 = Absorbance of mixture at wavelength 2

ay1 = Absorptivity of drug B at wavelength 1

ay2 = Absorptivity of drug B at wavelength 2

2. Material and method

2.1. Apparatus

A double beam UV-Visible spectrophotometer (Shimadzu model-1800, Software-UV Probe, Version 2.42) having two matched quartz cells with 1 cm light path.

2.2. Reference samples

CEF and LIN reference standard are kindly supply by Wockhardt, Mumbai and Aristo pharmaceuticals Pvt. Ltd, Mumbai as a gift sample respectively.

2.3. Pharmaceutical formulation

Linox*-XT tablet, labelled to contain 500 mg CEF and 600 mg LIN, manufactured by Unichem laboratories Ltd.

2.4. Materials and reagents

Methanol AR grade (RANKEM)

2.5. Standard solutions

2.5.1. Standard solution of Cefuroxime Axetil (CEF)

Accurately weighed quantity of CEF 100 mg was transferred to 100ml volumetric flask, dissolved and diluted up to mark with Methanol to give a standard stock solution having strength 1000µg/ml. Then pipette out 10ml from the standard stock solution in other 100ml volumetric flask and diluted up to mark with Methanol to give a working standard solution having strength 100µg/ml. Pipette out 25ml from the working standard solution in other 50ml volumetric flask and diluted up to mark with Methanol to give a working standard solution having strength 50µg/ml.

2.5.2. Standard solution of Linezolid (LIN)

Accurately weighed quantity of LIN 100 mg was transferred into 100 ml volumetric flask, dissolved and diluted up to mark with Methanol to give a stock solution having strength 1000µg/ml. Then pipette out 10ml from the standard stock solution in other 100ml volumetric flask and diluted up to mark with Methanol to give a stock solution having strength 100µg/ml. Pipette out 25ml from the working standard solution in other 50ml volumetric flask and diluted up to mark with Methanol to give a working standard solution having strength 50µg/ml.

2.5.3. Preparation of standard mixture

Pipette out accurately 0.4 ml of CEF stock solution (100µg/ml), 0.48 ml of LIN stock solution (100µg/ml) in 10 ml volumetric flask and make up the volume up to the mark with Methanol. It gives solution containing CEF 4µg/ml, LIN 4.8µg/ml.

2.5.4. Test Sample Preparation

Dissolve tablet sample in 100 ml volumetric flask containing 100 ml methanol. Take 1 ml tablet sample solution in 10ml volumetric flask and make up volume up to mark with methanol.

2.6. Procedures

2.6.1. Calibration curve for Cefuroxime Axetil

This series consisted of five concentrations of standard CEF solution ranging from 2-6µg/ml. The solutions were prepared by pipette out standard CEF working solution (0.4ml, 0.6ml, 0.8ml, 1ml, 1.2ml) was transferred into a series of 10 ml volumetric flasks and volume was adjusted up to mark with Methanol. A zero order spectra of the resulting solutions were recorded, measured the absorbance at 276.60nm against a reagent blank solution (Methanol). Calibration curve was prepared by plotting absorbance versus respective concentration of CEF.

2.6.2. Calibration curve for Linezolid

This series consisted of five concentrations of standard LIN solution ranging from 2.4-7.2µg/ml. The solutions were prepared by pipette out Standard LIN working solution (0.48ml, 0.72ml, 0.96ml, 1.20ml, and 1.44ml) was transferred into a series of 10 ml volumetric flasks and volume was adjusted up to mark with Methanol. A zero order spectra of the resulting solutions were recorded and measured the absorbance at 257.40 nm against a reagent blank solution (Methanol). Calibration curve was prepared by plotting absorbance versus respective concentration of LIN.

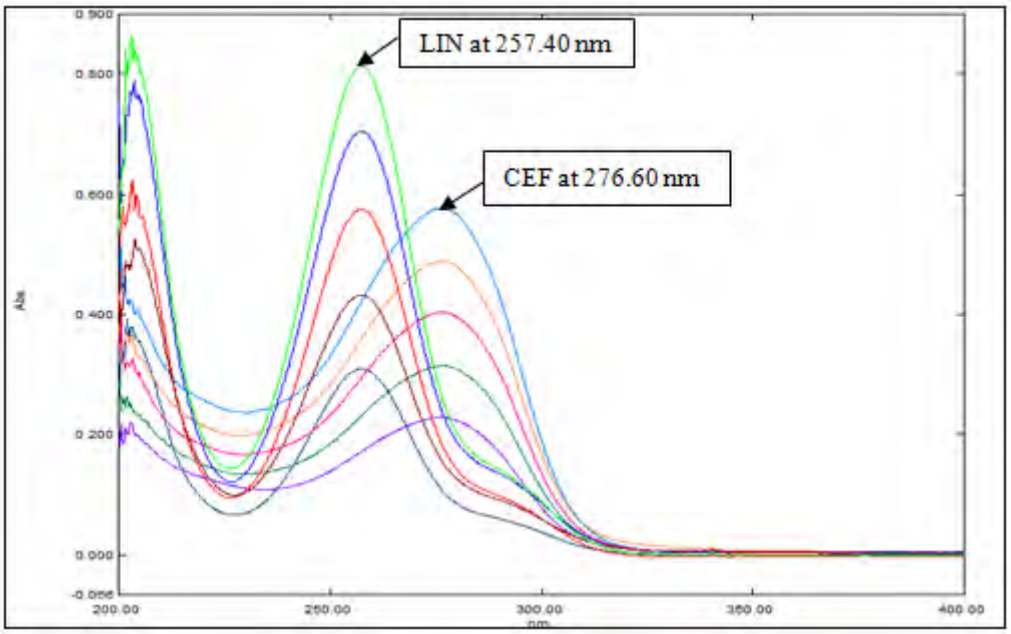

Fig. 2 Overlay linear spectra of CEF and LIN

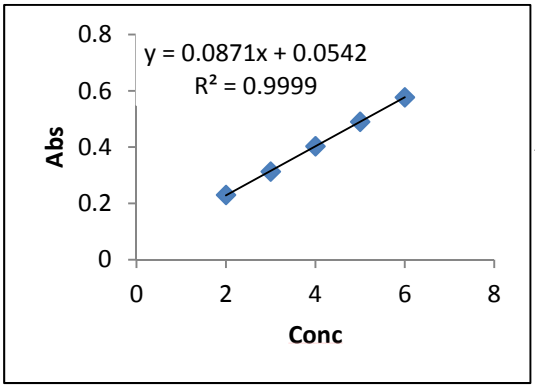

Fig. 3 calibration curve of CEF at 276.60nm

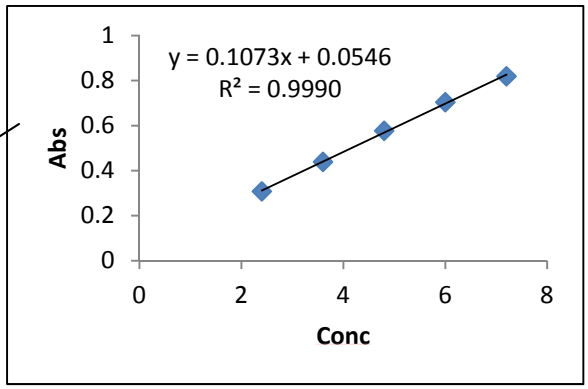

Fig. 4 calibration curve of LIN at 257.40nm

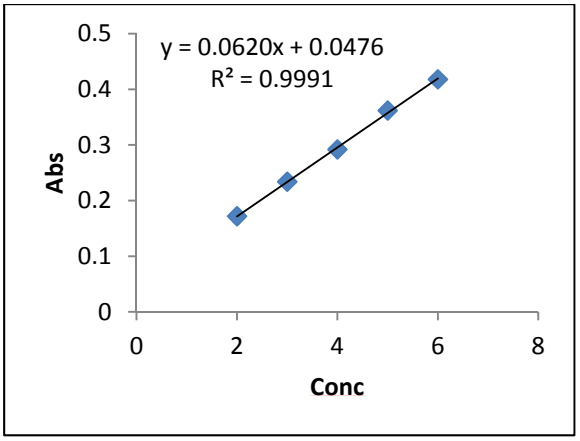

Fig. 5 calibration curve of CEF at 257.40nm

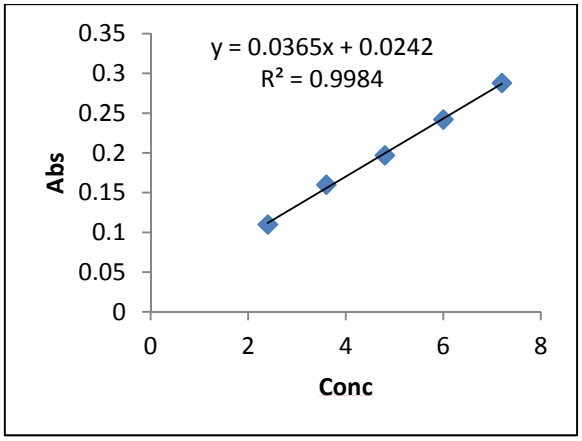

Fig. 6 calibration curve of LIN at 276.60nm

3. Experimental Work

3.1. Linearity and range

The linearity of method is its ability within a given range to obtain test results which are directly or through a mathematical transformation, proportional to the concentration of analyte. Linearity of the method was determined at five concentration levels for CEF and LIN independently.

3.2. Accuracy

The accuracy of an analytical method is the closeness of the test results to the true value. It was tested by spiking standard CEF solution in different concentration 80, 100 and 120% to a tablet solution. The tablet solution was analyzed at 276.60 nm for estimation of CEF. Similarly, the accuracy for LIN was determined at 257.40 nm, respectively.

3.3. Precision

The intra-day precision (repeatability) of method was determined by measuring the absorbance of tablet solution-I at 276.60 and 257.40 nm for CEF and LIN, respectively. The inter-day precision (intermediate precision) was determined by measuring the absorbance of tablet solution-I at 276.60 and 257.40 nm for CEF and LIN, respectively. The %RSD was calculated for intra and inter-day precision.

3.4. LOD and LOQ

The LOD of an analytical method is the lowest amount of analyte in a sample which can be detected but not necessarily quantified. The detection limit (DL) of method was determined by equation, DL = (3.3 σ)/S, where, σ– standard deviation of blank response, S– slope of the calibration curve. The quantitation limit (QL) of analyte was determined by equation DL = (10 σ)/S, where, σ– standard deviation of blank response, S– slope of the calibration curve.

4. Results and discussion

A) Linearity and Range:

The linearity range for CEF at 276.60 nm&257.40 nm and LIN at 257.40 nm&276.60 nm was found to be in the range of 2-6 µg/ml and 2.4-7.2 µg/ml respectively.

Tab.1 Linearity data for CEF at 276.60 nm

Sr. No	Conc. (µg/ml)	Absorbance at 276.60 nm Mean ± S.D. (n=5)	%R.S.D.
1	2	0.2300±0.000632	0.274
2	3	0.3131±0.001169	0.373
3	4	0.4031±0.000753	0.186
4	5	0.4901±0.001169	0.238
5	6	0.5770±0.000632	0.109

Tab.2 Linearity data for LIN at 257.40 nm

Sr. No	Conc. (µg/ml)	Absorbance at 257.40 nm Mean ± S.D. (n=5)	%R.S.D.
1	2.4	0.3081±0.000753	0.244
2	3.6	0.4390±0.000632	0.143
3	4.8	0.5770±0.001095	0.189
4	6	0.7043±0.001033	0.146
5	7.2	0.8191±0.001329	0.162

Tab.3 Linearity data for CEF at 257.40 nm

Sr. No	Conc. (µg/ml)	Absorbance at 276.60 nm Mean ± S.D. (n=5)	%R.S.D.
1	2	0.1726±0.000816	0.472
2	3	0.2340±0.001897	0.810
3	4	0.2921±0.001329	0.454
4	5	0.3620±0.001414	0.390
5	6	0.4186±0.001211	0.289

b.4 Linearity data for LIN at 276.60 nm

Sr. No	Conc. (µg/ml)	Absorbance at 257.40 nm Mean ± S.D. (n=5)	%R.S.D.
1	2.4	0.1101±0.000632	0.574
2	3.6	0.1601±0.000753	0.470
3	4.8	0.1970±0.000632	0.320
4	6	0.2421±0.001169	0.482
5	7.2	0.2880±0.001265	0.439

B) Precision:

I. Repeatability:

The data for repeatability for CEF at 276.60 nm&257.40 nm and LIN at 257.40 nm&276.60 nm is shown in table 5&6 respectively.

Tab.5 Repeatability data of CEF and LIN

Sample	Conc.	Mean ± S.D. (n=6)	%R.S.D.
Cefuroxime Axetil	4	0.40333 ± 0.000816	0.202%
Linezolid	4.8	0.57733 ± 0.003386	0.577%

Tab.6 Repeatability data of CEF and LIN

Sample	Conc.	Mean ± S.D. (n=6)	%R.S.D.
Cefuroxime Axetil	4	0.29216 ± 0.003488	1.193%
Linezolid	4.8	0.19700 ± 0.001414	0.717%

II. Intraday precision:

The data for intraday precision for CEF at 276.60 nm&257.40 nm and LIN at 257.40 nm&276.60 nm is shown in table 7, 8, 9& 10 respectively.

Tab.7 Intraday precision data of CEF at 276.60nm

Sr. No.	Conc. (µg/ml)	Mean abs. ± S.D. (n=3)	%R.S.D.
1.	3	0.313667±0.002082	0.663
2.	4	0.403333±0.000577	0.143
3.	5	0.490333±0.001528	0.311

Tab.8 Intraday precision data of LIN at 257.40nm

Sr. No.	Conc. (µg/ml)	Mean abs. ± S.D. (n=3)	%R.S.D.
1.	3.6	0.439000±0.002000	0.455
2.	4.8	0.577333±0.006506	1.126
3.	6	0.704000±0.007000	0.994

Tab.9 Intraday precision data of CEF at 257.40nm

Sr. No.	Conc. (µg/ml)	Mean abs. ± S.D. (n=3)	%R.S.D.
1.	3	0.234000±0.002000	0.854
2.	4	0.292000±0.003000	1.027
3.	5	0.362000±0.004000	1.104

Tab.10 Intraday precision data of LIN at 276.60nm

Sr. No.	Conc. (µg/ml)	Mean abs. ± S.D. (n=3)	%R.S.D.
1.	3.6	0.160000±0.002000	1.250
2.	4.8	0.197000±0.001000	0.507
3.	6	0.242000±0.002000	0.826

II. Interday precision:

The data for interday precision for CEF at 276.60 nm&257.40 nm and LIN at 257.40 nm&276.60 nm is shown in table 11, 12, 13& 14 respectively.

Tab.11 Interday precision data of CEF

Sr. No.	Conc. (µg/ml)	Mean abs. ± S.D. (n=3)	%R.S.D.
1.	3	0.313333±0.002517	0.803
2.	4	0.403000±0.002000	0.496
3.	5	0.490000±0.004000	0.816

Tab.12 Interday precision data of LIN

Sr. No.	Conc. (µg/ml)	Mean abs. ± S.D. (n=3)	%R.S.D.
1.	3.6	0.439333±0.005508	1.253
2.	4.8	0.577333±0.007506	1.300
3.	6	0.704333±0.009504	1.349

Tab.13 Interday precision data of CEF

Sr. No.	Conc. (µg/ml)	Mean abs. ± S.D. (n=3)	%R.S.D.
1.	3	0.313333±0.002517	0.803
2.	4	0.403000±0.002000	0.496
3.	5	0.490000±0.004000	0.816

Tab.14 Interday precision data of LIN

Sr. No.	Conc. (µg/ml)	Mean abs. ± S.D. (n=3)	%R.S.D.
1.	3.6	0.439333±0.005508	1.253
2.	4.8	0.577333±0.007506	1.300
3.	6	0.704333±0.009504	1.349

C) Accuracy:

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. The results are shown in table 15&16.

Tab.15 Determination of Accuracy for CEF and LIN

Drug	Level	Amount of sample (µg/ml)	Amount of standard spiked (µg/ml)	Total Amount (µg/ml)	Amount Recovered (µg/ml)	% Mean Recovery ± SD (n=3)
CEF (276.60 nm)	0 %	4	-	4	3.992	-
	80%	4	3.2	7.2	7.193	99.90%±0.005
	100 %	4	4	8	7.980	99.75%±0.017
	120 %	4	4.8	8.8	8.703	98.89%±0.081
LIN (257.40 nm)	0 %	4.8	-	4.8	4.784	-
	80 %	4.8	3.8	8.6	8.642	100.02%±0.009
	100%	4.8	4.8	9.6	9.500	98.95%±0.081
	120 %	4.8	5.7	10.5	10.543	99.83%±0.009

Tab.16 Determination of Accuracy for CEF and LIN

Drug	Level	Amount of sample (µg/ml)	Amount of standard spiked (µg/ml)	Total Amount (µg/ml)	Amount Recovered (µg/ml)	% Mean Recovery ± SD (n=3)
CEF (257.40 nm)	0 %	4	-	4	3.974	-
	80%	4	3.2	7.2	7.183	99.76%±0.016
	100 %	4	4	8	8.006	100.07%±0.016
	120 %	4	4.8	8.8	8.710	98.97%±0.088
LIN (276.60 nm)	0 %	4.8	-	4.8	4.816	-
	80 %	4.8	3.8	8.6	8.523	98.64%±0.096
	100%	4.8	4.8	9.6	9.710	101.14%±0.113
	120 %	4.8	5.7	10.5	10.514	99.56%±0.027

Analysis of marketed formulation: Applicability of the proposed method was tested by analyzing the commercially available tablet formulation Linox*-XT. The results are shown in table 17.

Tab.17 Analysis of marketed formulation

Formulation (Tablet Linox*-XT)	Actual concentration µg/ml		Amount obtained µg/ml		% CEF ± SD (n=3)	% LIN ± SD (n=3)
	CEF	LIN	CEF	LIN		
Batch No. MCRAE03	4	4.8	3.93	4.82	98.40± 0.011	100.43± 0.001

Tab.18 Summary of Validation parameter of proposed method

Parameter		CEF at 276.60 nm	LIN at 257.40 nm	CEF at 257.40 nm	LIN at 276.60 nm
Conc. range (µg/ml)		2-6	2.4-7.2	2-6	2.4-7.2
Regression equation (y = mx + c)		0.0871x+0.0542	0.1073x+0.0546	0.0620x+0.0476	0.0365x+0.0242
Mean of Slop (m) (n=6)		0.0871	0.1073	0.0620	0.0365
SD of Intercept (c) (n=6)		0.05430	0.05460	0.04790	0.02420
Regression co-efficient		0.9999	0.9990	0.9991	0.9984
Repeatability (%RSD) (n=6)		0.202	0.577	0.933	0.717
Intraday precision (n=3) (% RSD)		0.372	0.858	0.995	0.861
Interday precision (n=3) (% RSD)		0.705	1.300	1.159	1.376
LOD (µg/ml) (n=6)		0.026	0.021	0.065	0.972
LOQ (µg/ml) (n=6)		0.079	0.065	0.198	1.475
% Recovery	**80%**	99.90%	100.02%	99.76%	98.64%
	100%	99.75%	98.95%	100.07%	101.14%
	120%	98.89%	99.83%	98.97%	99.56%

5. Conclusion

From the overlay spectra of Cefuroxime Axetil and Linezolid it is observed that estimation of both the drug can be possible using Simultaneous Equation method. The method was developed and validated. The value of % recovery and standard deviation reveals that the proposed method was successfully utilized for the estimation of Cefuroxime Axetil and Linezolid in tablet dosage form. A simple, rapid and sensitive method is proposed for the analysis of two binary mixtures with overlapping spectra. The method involves the generation of absorbance spectra followed by measurement of the absorbance. Therefore, the presented methodology is adequate for the routine quality control analysis of these fixed-dose combinations.

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