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Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Cefuroxime Axetil and Linezolid in Tablet Dosage Form

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ABSTRACT:

To develop simple, sensitive, accurate, rapid and precise first derivative spectrophotometric method for the simulataneous estimation of Cefuroxime Axetil (CEF) and Linezolid (LIN) in Tablet dosage form. The derivative spectrophotometric method was based on the determination of both the drugs at their respective zero crossing point (ZCP). The first order derivative spectrum was obtained in methanol and the determinations were made at 257.40 nm (ZCP of Linezolid) for Cefuroxime Axetil and 276.60 nm (ZCP of Cefuroxime Axetil) for Linezolid. The linearity was obtained in the concentration range 2-6 µg/ml for Cefuroxime Axetil and 2.4-7.2 µg/ml for Linezolid. The results of analysis have been validated statistically and by recovery studies.

Key words: Cefuroxime Axetil (CEF), Linezolid (LIN), First Order Derivative Method.

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INTRODUCTION:

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 active against Gram-negative bacteria. 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.
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. Linezolid is chemically N-[(5S)-3-[3-fluoro-4-(4-morpholinyl) phenyl]-2-oxo-5-oxazolidinyl] methyl]acetamide. Combination of Cefuroxime Axetil and Linezolid is used to treat bacterial infection.(1-5) The aim of the present work was to develop a new simple, rapid, selective method for the simultaneous determination of components having overlapping spectra in binary mixtures. The first order derivative method for simulataneous determinations were made at 257.40 nm (ZCP of LIN) for CEF and 276.60 nm (ZCP of CEF) for LIN.(6-16)

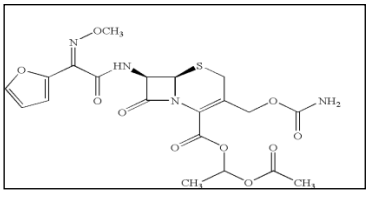

Figure 1: Structure of Cefuroxime Axetil

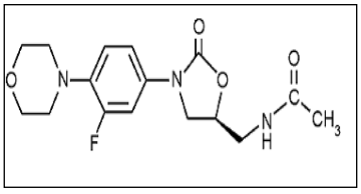

Figure: 2 Structure of Linezolid

MATERIALS AND METHODS:

Instruments and Apparatus:

A double beam UV-Visible spectrophotometer, SHIMADZU (model UV-1800, Software-UV Probe, Version 2.42) having two matched quartz cells with 1 cm light path. All weighing was done on Reptech electronic analytical balance. All the apparatus used were calibrated.

Reagents and Chemicals:

Cefuroxime Axetil (Gift sample, Wockhardt,Mumbai) and Linezolid (Gift sample, Aristo pharmaceuticals Pvt. Ltd,Mumbai). Methanol AR grade (Rankem)

Marketed Formulation:

Linox*-XT tablet manufactured by Unichem laboratories Ltd.

Preparation of Standard solution:

Cefuroxime Axetil (CEF) standard stock solution:

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.

Linezolid (LIN) standard stock solution:

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.

Selection of Analytical Wavelength:

2-6 µg/ml solutions of CEF were prepared in Methanol and spectrum was recorded between 200-400 nm. Spectrums for above concentration were obtained with n=5 and 2.4-7.2 µg/ml solutions of LIN were prepared in Methanol and spectrum was recorded between 200-400nm. CEF at 257.40 nm(ZCP of LIN) and LIN at 276.44 nm(ZCP of CEF). The overlay spectrums of CEF and LIN at different concentration were recorded.

Method:

Calibration curve for the CEF (2 - 6 µg/ml)

Appropriate volume of aliquot from standard CEF stock solution was transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with the methanol to obtain concentration of 2, 3, 4, 5, and 6µg/ml. The curve of each solution against the Methanol was recorded. Absorbance of CEF at 257.40 nm (ZCP of LIN) was measured and the plot of absorbance vs. concentration was plotted.

Calibration curve for the LIN (2.4 - 7.2 µg/ml)

Appropriate volume of aliquot from standard LIN stock solution was transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with the methanol to obtain concentration of 2.4, 3.6, 4.8, 6, and 7.2µg/ml. The curve of each solution against the Methanol was recorded. Absorbance of LIN at 276.60 nm (ZCP of CEF) was measured and the plot of absorbance vs. concentration was plotted.

Preparation of Sample solution:

Take twenty tablets were weighed and finely powdered. The powder equivalent to 500 mg CEF and 600 mg LIN was accurately weighed and transferred to volumetric flask of 100ml capacity. Powder was dissolved in methanol in volumetric flask. The flask was shaken and volume was made up to the mark with methanol. The solution was filtered through whatmann filter paper. Then 10 ml of solution was taken and transferred to volumetric flask of 100 ml and volume was made up to the mark with methanol. Further 0.4 ml of this solution was transferred to volumetric flask of 10ml capacity. Volume was made up to the mark with methanol to give a solution containing 4µg/ml CEF and 4.8µg/ml LIN. This solution was used for the estimation of CEF and LIN in tablet dosage form.

Estimation of CEF and LIN by First order derivative Method.

Absorbance of the resulting solution was measured at 257.40 nm for determination of CEF (ZCP of LIN) and at 276.60 nm for determination of LIN (ZCP of CEF).

Method Validation:

1. Accuracy

Accuracy was determined by calculating recovery of CEF and LIN by the standard addition method. Known amounts of standard solutions of CEF and LIN were added to test solutions. The recovery was calculated by measuring absorbance.

2. Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples.

3. Repeatability

Standard solutions of CEF and LIN were prepared of linearity range and spectrums were recorded. Absorbance was measured at 257.40 nm and 276.60 nm.

4. Intraday and Inter day Precision

Variations of results within the same day (intraday), variation of results between days (inter day) were analyzed.

Intraday precision was determined by analyzing CEF and LIN individually for three times in the same day at 257.40 nm and 276.60 nm.

Inter day precision was determined by analyzing CEF and LIN individually daily for three day at 257.40 nm and 276.60 nm

5. Linearity

The linearity of analytical method is its ability to test results that are directly proportional to the concentration of analyte in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity.

6. RESULT AND DISCUSSION:

The Linearity range for CEF and LIN was found to be in the range of 2-6 µg/ml and 2.4-7.2 µg/ml respectively. The method was determined at five concentration levels for CEF at 257.40 nm (ZCP of LIN) and LIN at 276.60 nm (ZCP of CEF) independently.

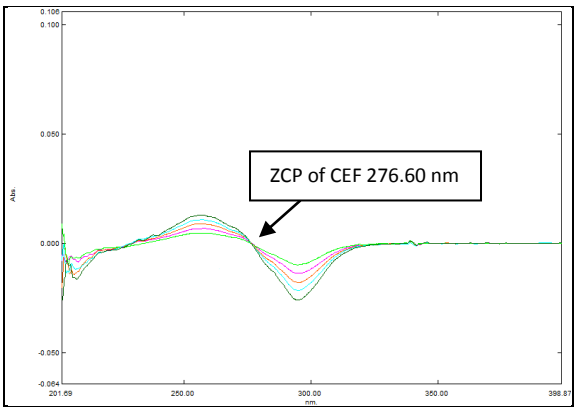

Figure: 3 Overlay Spectra of CEF at (2-6 µg/ml)

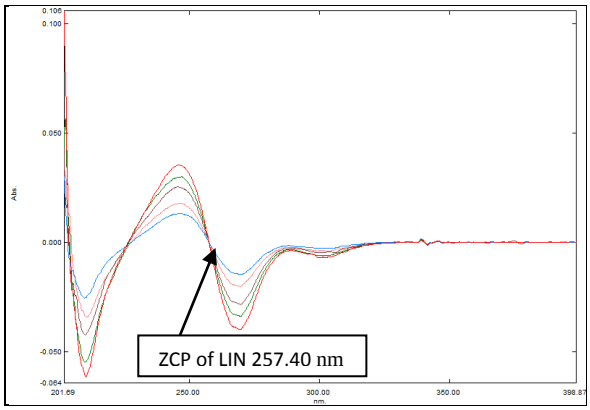

Figure: 4 Overlay Spectra of LIN at (2.4-7.2 µg/ml)

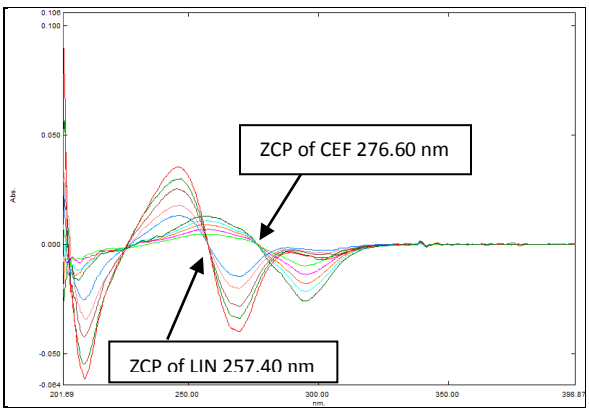

Figure: 5 Overlay spectra of CEF and LIN

Table: 1 Linearity data for CEF at 257.40 nm (ZCP of LIN)

Concentration (µg/ml)	Absorbance at 257.40 nm Mean± S.D. (n=5)
2	0.0050 ± 0.00010
3	0.0069 ± 0.00012
4	0.0087 ± 0.00016
5	0.0108 ± 0.00016
6	0.0128 ± 0.00016

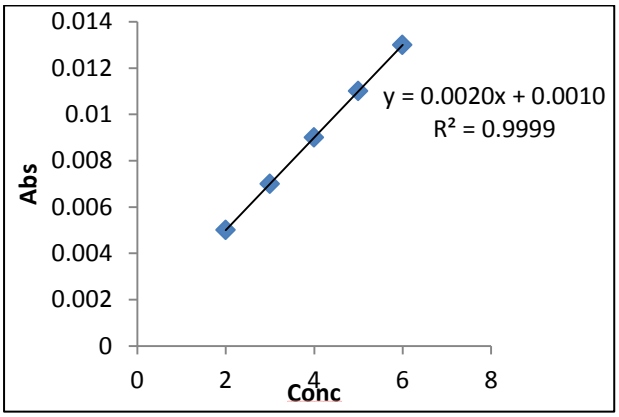

Figure: 6 Calibration curve of CEF at 257.40 nm

Table: 2 Linearity data for LIN at 276.60 nm (ZCP of CEF)

Concentration (µg/ml)	Absorbance at 223.38 nm Mean ± S.D. (n=5)
2.4	0.0079 ± 0.00012
3.6	0.0116 ± 0.00016
4.8	0.0157 ± 0.00026
6	0.0182 ± 0.00030
7.2	0.0228 ± 0.00040

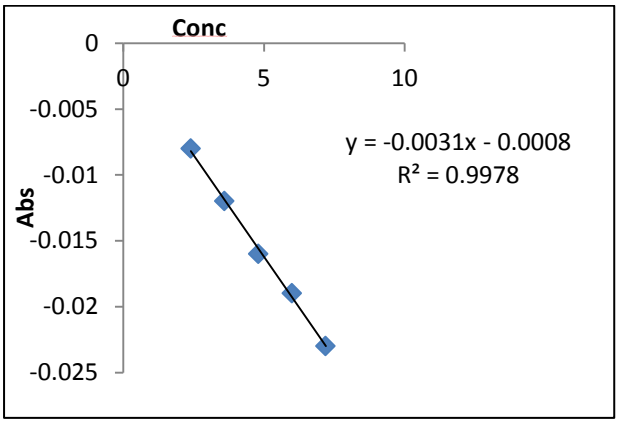

Figure: 7 Calibration curve of LIN at 276.60 nm

Table: 3 Intraday precision

Drugs	Conc. (µg/ml)	Absorbance Mean ± S.D. (n=3)	%RSD
CEF	3	0.0070 ± 0.00010	1.428
	4	0.0088 ± 0.00015	1.732
	5	0.0111 ± 0.00010	0.900
LIN	3.6	-0.0119 ± 0.00010	0.840
	4.8	-0.0164 ± 0.00017	1.054
	6	-0.0188 ± 0.00011	0.609

Table: 4 Interday precision

Drugs	Conc. (µg/ml)	Absorbance Mean ± S.D. (n=3)	%RSD
CEF	3	0.0069 ± 0.00011	1.658
	4	0.0089 ± 0.00017	1.943
	5	0.0111 ± 0.00017	1.558
LIN	3.6	-0.0118 ± 0.00020	1.600
	4.8	-0.0162 ± 0.00025	1.549
	6	-0.0188 ± 0.00015	0.812

Table: 5 Accuracy study for CEF and LIN

Drugs	Level	Amt. of Sample (µg/ml)	Amt. of STD Spiked	Total Amt.	Amt. Found (µg/ml)	% Recovery
CEF	80	4	3.2	7.2	7.30	101.38
	100	4	4	8	7.91	98.95
	120	4	4.8	8.8	8.66	98.48
LIN	80	4.8	3.8	8.6	8.55	99.06
	100	4.8	4.8	9.6	9.58	99.79
	120	4.8	5.7	10.5	10.60	100.38

Table: 6 Analysis of marketed formulation

Formulation (Tablet Linox*-XT)	Actual conc. (µg/ml)		Amt. obtained (µg/ml)		%CEF ± SD (n=3)	%LIN ± SD (n=3)
	CEF	LIN	CEF	LIN		
Batch No. MCRAE03	4	4.8	4.10	4.72	101.5± 0.020	98.33± 0.010

CONCLUSION:

From the overlay spectra of Cefuroxime Axetil and Linezolid it is observed that estimation of both the drug can be possible using First order derivative 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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