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DEVELOPMENT AND VALIDATION OF FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF CEFUROXIME AXETIL AND LINEZOLID IN COMBINED DOSAGE FORM

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Abstract

The present manuscript describes simple, sensitive, rapid, accurate, precise and cost effective First derivative spectrophotometric method for the simultaneous estimation Cefuroxime Axetil and Linezolid in combines dosage form. The first order derivative absorption at 276.84 nm (zero crossing point for Cefuroxime axetil) was used for Linezolid and 257.78 nm (zero crossing point for Linezolid) was used for Cefuroxime axetil. The linearity was obtained in the concentration range of 5-25 µg/ml for Cefuroxime Axetil and 6-30 µg/ml for Linezolid with correlation coefficient (R^2) 0.996 and 0.998, respectively. The mean % recoveries were found to be in the range of % and 98.93-101.53 % for Cefuroxime Axetil and Linezolid, respectively. The suitability of these methods for the quantitative determination of Cefuroxime Axetil and Linezolid was proved by validation. The proposed method has been validated as per ICH guideline and successfully applied to the simultaneous estimation of Cefuroxime Axetil and Linezolid in combined dosage form. The results of analysis have been validated statistically and by recovery studies.

Keywords: Cefuroxime Axetil, Linezolid, First order derivative, combined dosage form, Validation method.

Introduction

Cefuroxime Axetil is Chemically 1-acetyloxyethyl (6R,7R)-3-(carbamoyloxymethyl)-7-[[(2Z)-2-(furan-2-yl)-2-methoxyiminoacetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate[1]. Cefuroxime Axetil which is a potent antibiotic agent now a day's recommended as the oral therapy for bacterial infections. It belongs to cephalosporin family which has main effect to decrease bacterial infections, by interrupting the bacterial cell wall formation[2]. Linezolid is chemically N-(((5S)-3-(3-Fluoro-4-morpholinophenyl)-2-oxo-5-oxazolidinyl) methyl) Acetamide [1]. Linezolid as a oxazolidinone reduces growth of bacteria and helps in prevention of bacterial infections

[3]. Cefuroxime Axetil and Linezolid used in high bacterial infection like kidney, urinary tract, meningitis, respiratory tract infections and Pneumonia, skin infections and infections [4] . Cefuroxime Axetil is official in IP [5], USP-NF [6] and NF [7]. Linezolid is official in IP [5] (From Literature Survey, various method (UV, HPLC, HPTLC, GC and Colorimetric) were reported for the analysis of individual drug in combination with other drug but no method were reported for simultaneous estimation of Cefuroxime Axetil and Linezolid. Hence, the purpose of the present work was to develop and validate first order derivative Spectrophotometric method for simultaneous estimation of Cefuroxime Axetil and Linezolid in combined dosage form

Material and methods

Instruments

Spectrophotometric measurements were performed on Shimadzu UV –visible double beam spectrophotometer (Model- 1800). All weighing were done on electronic analytical balance (Wensar Dab220).

Chemicals and Reagents

The bulk drug, Cefuroxime Axetil was obtained from Centurion Laboratories, Baroda and Linezolid was obtained from nirlife Ltd, Ahmedabad. Fixed dose of combined dosage form of Cefuroxime Axetil 500 mg and Linezolid 600 mg were prepared in laboratory scale as pilot batch. Analytical grade methanol was procured from Merck Fine chemicals (Mumbai).

Selection of a Solvent: Methanol was selected as solvent for studying spectral characteristic of drugs.

Preparation of Standard Stock Solution

Accurately weighed 10 mg Cefuroxime Axetil and 10 mg Linezolid standard were transferred to separate 100 ml volumetric flask and dissolved in 100 ml methanol. The flasks were shaken and volume was made up to the mark with Methanol to give solution containing 100µg/ml Cefuroxime Axetil and Linezolid.

Preparation of Working Standard Solution

Cefuroxime Axetil and Linezolid From above solution of Cefuroxime Axetil pipette out 0.5, 1.0, 1.5, 2.0, 2.5 ml of the stock solution were further diluted to 10 ml volumetric flasks individually with methanol to get concentrations 5, 10, 15, 20, 25µg/ml. From above solution of Linezolid pipette out 0.6, 1.2, 1.8, 2.4, 3.0 ml of the stock solution were further diluted to 10 ml volumetric flasks individually with methanol to get concentrations 6, 12, 18, 24, 30 µg/ml.

Selection of Analytical Wavelength

Standard 5-25 µg/ml solutions of Cefuroxime Axetil and 6-30 µg/ml solutions of Linezolid were prepared in

methanol by appropriate dilution and spectrum was recorded between 200-400 nm. All zero order spectrum (D^0) were converted to first derivative spectrum (D^1) using delta lambda 2.0 and scaling factor 1. The overlain first derivative spectrums of Cefuroxime Axetil and Linezolid at different concentration were recorded. The zero crossing point (ZCP) of Cefuroxime Axetil was found to be 257.79 nm and ZCP of Linezolid was found to be 276.84 nm.

Assay of combined dosage form

The quantity of synthetic mixture powder equivalent to 10 mg of Cefuroxime Axetil and 12 mg of Linezolid was transferred in to 100 ml volumetric flask, containing methanol. The volume was made up to the mark with methanol and the solution filtered through 0.45µm Whatmann filter paper. An aliquot of this solution (1.0 ml from Cefuroxime Axetil stock solution and 1.2 ml from Linezolid stock solution) was transferred in to 10 ml volumetric flask and volume was made up to the mark with methanol to obtain final concentration of 10 µg/ml Cefuroxime Axetil and 12 µg/ml Linezolid. Absorbance of a sample solution recorded using first order derivative spectroscopy at 276.84nm (ZCP of Cefuroxime axetil) and 257.79 nm (ZCP of Linezolid) for determination of Cefuroxime Axetil and Linezolid respectively. The analysis procedure was repeated three times with combined dosage form.

Method Validation

Method validation was performed following ICH guidelines. The proposed method has been extensively validated in terms of linearity, accuracy and precision, limit of detection and limit of quantification.

Linearity (Calibration curve)

Appropriate volume of aliquot from Cefuroxime Axetil and Linezolid standard stock solution was transferred to 10 ml volumetric flask. The volume was made up to the mark with methanol to give solution containing 5-25 µg/ml Cefuroxime Axetil and 6-30 µg/ml Linezolid. All D^1spectrums were recorded using above Spectrophotometric condition. D^1 absorbance at 276.84 nm and 257.79 nm were recorded for, Cefuroxime Axetil and Linezolid respectively (n=6). Calibration curve were cssonstructed by plotting average absorbance versus concentrations for both drugs. Straight line equations were obtained from these calibration curves. The linear regression equation of Cefuroxime Axetil was $y = 0.001x + 0.001$ ($R^2 = 0.996$) and Linezolid was $y = -0.001x + 0.001$ ($R^2 = 0.998$).

Accuracy

Accuracy was assessed by determination of the recovery of the method by addition of standard drug to the prequantified sample preparation at three different concentration levels 80 %, 100 % and 120 %, taking in to consideration percentage purity of added drug sample. The amounts of Cefuroxime Axetil and Linezolid were

estimated by applying obtained values to the respective regression line equations. Each concentration was analyzed 3 times and average recoveries were measured.

Precision: The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the method was verified as repeatability, intra-day, inter-day and reproducibility.

Table-1: Regression analysis data and summary of validation parameters for the proposed method.

Parameter	**First-Derivative UV Spectrophotometric**	
	Cefuroxime axetil	Linezolid
Concentration range (µg/ml)	5-25	6-30
Regression equation	y = 0.001x + 0.001	y = -0.001x - 0.001
Slope	-0.001	-0.001
Intercept	0.001	-0.000
Correlation Coefficient (R^2)	0.996	0.998
Accuracy (% recovery, n=3)	99.0-101.11	98.93-100.66
Repeatability (%RSD, n=6)	1.66	1.4477
Intraday (%RSD, n=3)	1.491715-1.60309	0.9039-1.46115
Interday (%RSD, n=3)	1.560469-1.825199	1.20368-1.58032
LOD (µg/ml)	5.77	2.81
LOQ (µg/ml)	17.48	8.52

The repeatability was evaluated by assaying 6 times of sample solution of 15 µg/ml Cefuroxime Axetil and 18 µg/ml Linezolid prepared for assay determination without changing the parameter. The intra-day and inter-day precision study of Cefuroxime Axetil and Linezolid was carried out by estimating different concentration of Cefuroxime Axetil (10, 15, 20 µg/ml) and Linezolid (12, 18, 24 µg/ml), 3 times on same day and on 3 different day (first, second and third).

Limit of Detection (LOD) and Limit of Quantification (LOQ)

ICH guideline describes several approaches to determine the detection and quantification limits. These include visual evaluation, signal-to-noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and were calculated according to the 3.3 × (SD/Slope) and 10 × (SD/Slope) criteria, respectively; where SD is the standard deviation of y-intercept of regression line and S is the slop of the calibration curve.

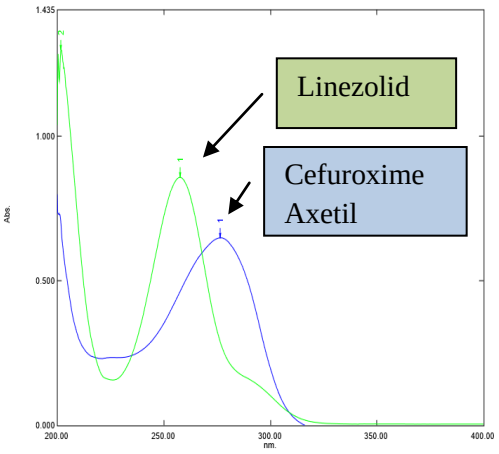

Figure 3: UV Spectrum for Cefuroxime Axetil (15 µg/ml) at 276.84 nm and Linezolid (18 µg/ml) at 257.79 nm in Methanol.

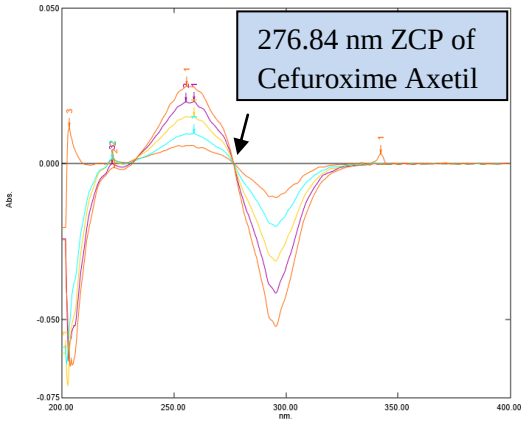

Figure 4: D^1 spectrum of Cefuroxime Axetil (5-25 µg/ml) in Methanol.

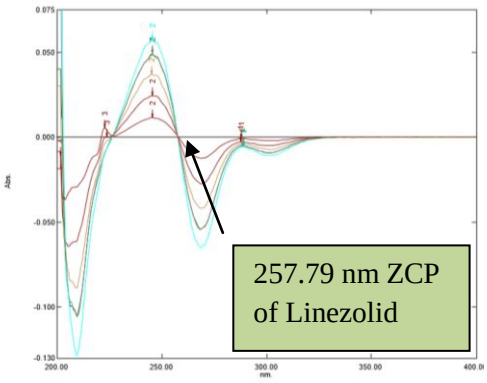

Figure 5: Overlain D^1 spectrum of Linezolid (6-30 µg/ml) in Methanol.

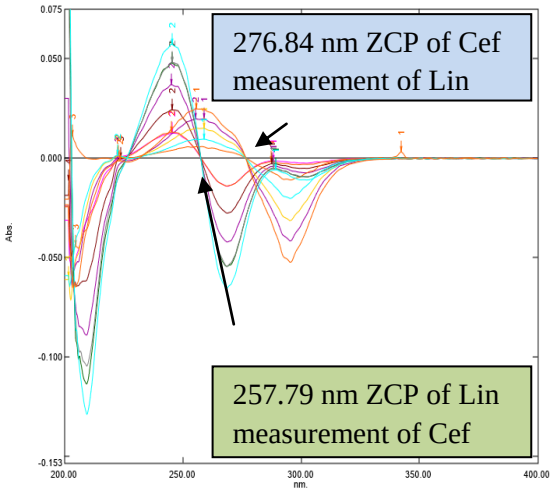

Figure 6: Overlain D^1 spectrum of Cefuroxime Axetil(Cef) (5-25 µg/ml) and Linezolid(Lin) (6-30 µg/ml) in Methanol.

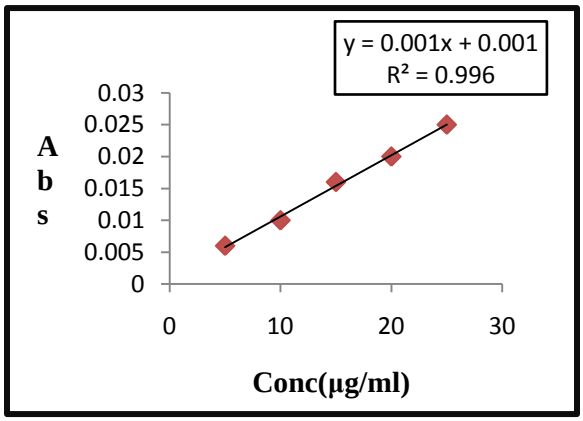

Figure 7: Calibration curve of Cefuroxime Axetil at 276.84 nm in Methanol.

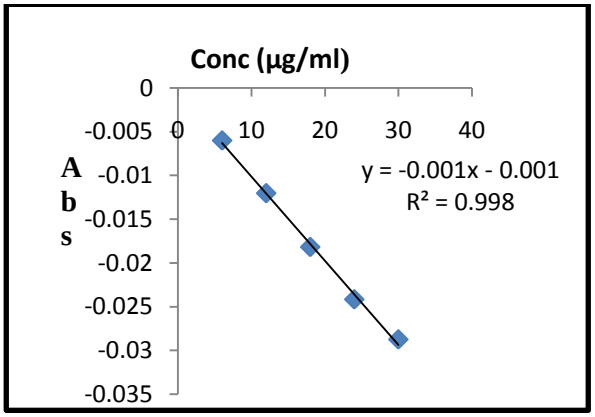

Figure 8: Calibration curve of Linezolid at 257.79 nm in Methanol.

Result and Discussion

A reliable first order derivative Spectrophotometric method was developed for simultaneous estimation of Cefuroxime Axetil and Linezolid in synthetic mixture by UV Spectrophotometric. Beers law was obeyed in concentration range of 5-25 µg/ml for Cefuroxime Axetil and 6-30 µg/ml for Linezolid at 276.84nm and 257.79 nm wavelengths, respectively. The correlation coefficient Cefuroxime Axetil and Linezolid was found to be R^2= 0.996 and 0.998. The mean % recoveries were found to be in the range of % and 98.93-101.53 %, respectively. Precision (% RSD) of Cefuroxime Axetil and Linezolid was found to be 1.400-1.711 % and 0.426-1.825%, respectively. The LOD and LOQ were 5.77µg/ml and 17.48µg/ml of Cefuroxime Axetil and 2.81µg/ml and 8.52µg/ml of Linezolid, respectively. The proposed method was precise, accurate and reproducible and acceptable recovery of the analyzes, which can be applied for the analysis of Cefuroxime Axetil and Linezolid in synthetic mixture.

Table-2: Recovery data of proposed method.

Drug	Level (%)	Test amount (µg/ml)	Spiked STD Amount (µg/ml)	Total Amount Recovered (µg/ml)	%Mean recovery ± RSD. (n=3)
Cefuroxime axetil	80	10	8	18.2	101.11 ± 0.7928
	100	10	10	20.1	100.50 ± 1.8567
	120	10	12	21.8	99.000 ± 1.4181
Linezolid	80	12	9.6	21.37	98.93 ± 1.5044
	100	12	12	24.13	101.66 ± 0.9569
	120	12	14.4	26.13	101.53 ± 0.8837

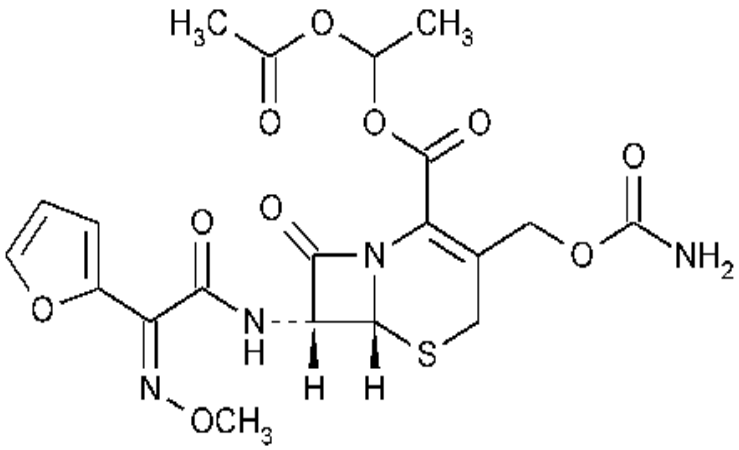

Figure 1: Structure of Cefuroxime Axetil [1]

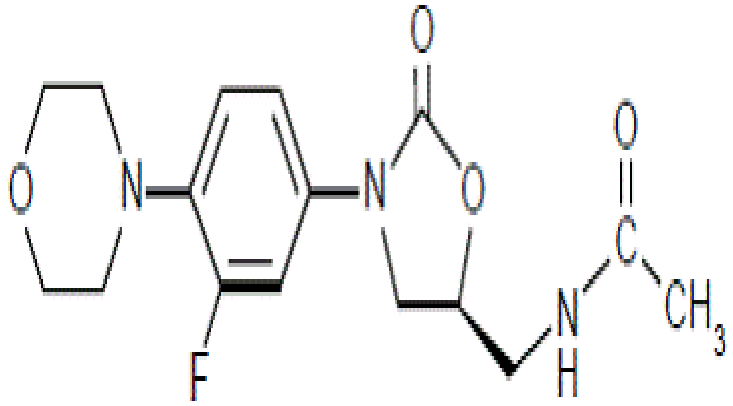

Figure 2: Structure of Linezolid [1]

Conclusion

The results of present study indicate that the proposed UV spectroscopic method is simple, rapid, precise and accurate. The developed UV spectroscopic method was found suitable for determination of Cefuroxime Axetil and Linezolid in bulk drug and combined dosage form without any interference from the excipients. Statistical analysis proves that the method is repeatable and selective for the analysis of Cefuroxime Axetil and Linezolid in combination. It can therefore be concluded that the developed analytical method is precise & accurate and can be use for routine Analysis of both the drug in combination.

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