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Research Article

Simultaneous Estimation of Cefuroxime Axetil and Linezolid by Three Novel Spectrophotometric Methods in Pharmaceutical Dosage Form and their Comparison Using ANOVA

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ABSTRACT
Three simple, accurate, sensitive, precise and economical UV spectrophotometric methods (A, B & C) have been developed for simultaneous estimation of cefuroxime axetil and linezolid in pharmaceutical dosage form and their comparision using ANOVA. Method A employs solving of simultaneous equations based on the measurement of absorbance at two wavelengths, 277 nm and 257 nm which are the λ_{max} values of cefuroxime axetil and linezolid, respectively in methanol. Method B is based on the principle of Q- absorbance ratio where in the absorbance was measured at 272 nm (iso-absorptive point) and 257 nm (λ_{max} of linezolid) in methanol. The linearity was obtained in the concentration ranges of 3-11 and 3.6 – 13.2 µg/ml, respectively. Method C is based on the first derivative spectrophotometric method at zero crossing wavelengths. In this method the zero crossing point of cefuroxime axetil was selected at 256 nm and for linezolid it was 275 nm. The linearity was obtained in the concentration range of 10-30 µg/ml for cefuroxime axetil and 12-36 µg/ml for linezolid. The accuracy and precision of the methods were determined and validated statistically. All the methods showed good reproducibility and recovery with % RSD less than 2. The three methods were compared using one -way ANOVA and the f_{cal} value was found to be less than f_{tab} value indicating that there is no significant difference in the assay results by the three methods. All methods were found to be rapid, specific, precise and accurate and these methods require no preliminary separation and found no interferences from the tablet excipients so it can be used for routine analysis of both drugs in quality control laboratories.

Keywords: Cefuroxime axetil, Linezolid, Simultaneous equation, Q-absorbance ratio, First derivative spectrophotometric, validation.

INTRODUCTION

Cefuroxime axetil (CEF) is a second generation oral cephalosporin antibiotic used for acute otitis media, bone and joint infections, meningitis, pharyngitis and tonsilitis, respiratory tract infections, septicemia, skin and skin structure infections[1,2]. It is chemically(6R, 7R) – 3 – carbamoyloxymethy l – 7 - [(Z)-2-(2-furyl) – 2 - (methoxyimino) acetamido] – ceph – 3 - em-4- carboxylic acid[3] (Figure 1) .CEF is official in Indian Pharmacopoeia (IP)[4], British Pharmacopoeia (BP)[5] and United State Pharmacopoeia (USP)[6]. IP, BP and USP describes liquid chromatography for the estimation of CEF. Linezolid (LZD) is an oxazolidinone antibiotic (anti-bacterial) which inhibits bacterial protein synthesis[7]. It is chemically N{[(5S-)-3-[3-fluoro-4-(morphilin-4- yl) phenyl] -2-oxo-1,3-oxozolidin-5yl] methyl] acetamide}(Figure 2)[7]. The deep literature survey reveals that various spectrophotometric and chromatographic methods[8-22] available for the estimation of CEF and LZD alone and in combination with other drugs and in biological fluids. Combination of cefuroxime axetil and linezolid is not official in any pharmacopoeias and hence no official method available for analysis of both drugs in combination. Literature survey also reveals that there are no reported methods available for simultaneous estimation of CEF and LZD in combined dosage form. Therefore, simple, rapid, and reliable method for simultaneous estimation of these drugs in nasal drops seemed to be necessary. Spectrophotometric methods of analysis are more economic and simpler, compared to methods such as chromatography and electrophoresis. The purpose of this study was to determine both drugs concurrently by simple, accurate, rapid and precise simultaneous equation, Q-absorbance ratio and first derivative spectrophotometric assays for routine analysis.

MATERIALS AND METHODS

Apparatus and instrument

UV visible double beam spectrophotometer (SHIMADZU -1800, Japan) with software UV Probe 2.33, with spectral slit width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1cm matched quartz cells

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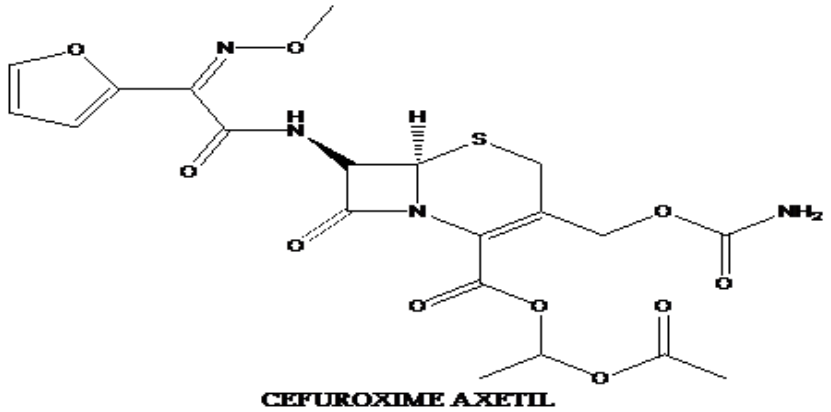

Figure 1: Chemical structure of cefuroxime axetil

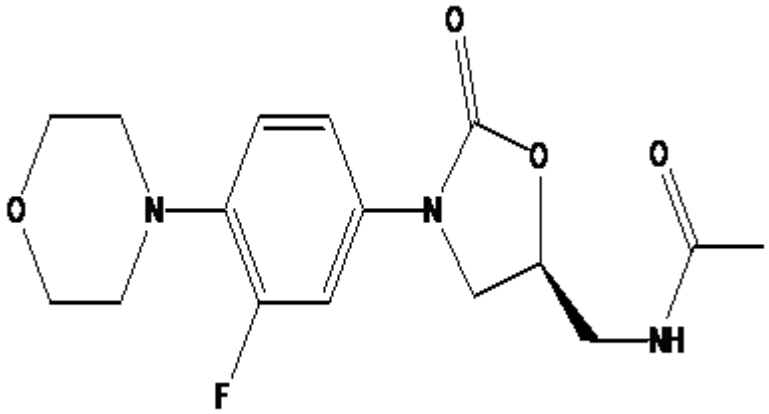

Figure 2: Chemical structure of linezolid

Table 1: Assay results for tablets using the proposed methods

Formulation	Proposed methods	Label Claim (mg)		Amount of drug found (mg)		% Label Claim Assay (n^a=3) ± SD^b	
		CEF	LZD	CEF	LZD	CEF	LZD
Tablets	METHOD A	500	600	496.33	594.66	98.66±0.55	99.11±0.25
	METHOD B	500	600	495.33	597.83	99.06±0.27	99.63±0.19
	METHOD C	500	600	493.33	596.33	99.26±0.48	99.38±0.40

Table 2: Application of the standard addition technique to the analysis of CEF and LZD in tablets by the proposed methods

Proposed methods	Concentration of drug taken (µg/ml)		Concentration of drug added (µg/ml)		Concentration of drug found (µg/ml)		% Recovery (n^a=3) ± SD^b	
	CEF	LZD	CEF	LZD	CEF	LZD	CEF	LZD
METHOD A	5	6	2.5	3	7.5	9	99.46±0.23	99.44±1.92
	5	6	5	6	10	12	99.53±0.11	99.44±1.92
	5	6	7.5	9	12.5	15	99.53±0.11	100.55±1.91
METHOD B	5	6	2.5	3	7.5	9	99.26±0.11	100.50±1.33
	5	6	5	6	10	12	99.40±0.20	99.44±1.92
	5	6	7.5	9	12.5	15	99.50±0.21	100.55±0.81
METHOD C	15	18	7.5	9	22.5	27	99.86±0.90	99.63±0.75
	15	18	15	18	30	36	99.66±0.35	100.36±0.80
	15	18	22.5	27	37.5	45	99.70±0.80	100.43±0.57

and digital balance Shimadzu ATX 224, Japan and ultrasonicator were used. Volumetric flasks and pipettes of borosilicate glasses were used in the study.

Chemicals and reagents

Pure drugs samples of cefuroxime axetil and linezolid were kindly supplied as a gift sample from Bharat

Table 3: Summary of validation parameter by developed method

Methods	Drug	Parameters							
		Slope	intercept	Correlation coefficient	LOD µg/ml	LOQ µg/ml	Interday (*n* = 3) (RSD , %)	Intraday (n = 3) (RSD , %)	Repeatability (RSD, %)
METHOD A	CEF (277 nm)	0.0374	0.0116	0.9998	0.11	3.0	0.16 -0.77	0.21 -0.77	0.75
	CEF (257nm)	0.0273	0.0067	0.9994	0.13	3.0	0.22 -1.07	0.22 -0.75	0.61
	LZD (277nm)	0.0199	0.0067	0.9971	0.19	3.6	0.67 -1.24	0.67 -1.90	0.32
	LZD (257 nm)	0.0648	0.0103	0.9993	0.16	3.6	0.21 -0.67	0.21 -0.67	0.21
METHOD B	CEF (272 nm)	0.0366	0.0109	0.9995	0.12	3.0	0.21 -1.08	0.30 -1.06	0.62
	CEF (257 nm)	0.0273	0.0067	0.9994	0.10	3.0	0.45 -0.80	0.40 -0.75	0.36
	LZD (272 nm)	0.0312	0.0079	0.9998	0.16	3.6	0.30-0.43	0.30-0.63	0.40
	LZD (257 nm)	0.0648	0.01030	0.9993	0.10	3.6	0.17-0.39	0.21-0.45	0.19
METHOD C	CEF (275 nm)	0.0015	0.0108	0.9995	0.14	10	0.23-0.47	0.33-0.49	0.50
	LZD (256 nm)	0.004	0.0306	0.9997	0.15	12	0.06-0.11	0.08-0.28	0.16

Pareneterals ltd, Vadodara and Alembic Pharmaceuticals ltd, Vadodara, respectively. Methanol (AR Grade) and other reagent were provided by Department of Quality Assurance, Pioneer Pharmacy Degree College, Vadodara, Gujarat, India. Marketed formulation (tablets containing CEF 500 mg and LZD 600 mg) was purchase from local market.

Selection of common solvent

Methanol of analytical reagent grade was selected as a common solvent for developing spectral characteristics of both drugs. The selection was made after assessing the solubility of both drugs in different solvents like water, chloroform, ether etc.

Preparation of standard stock solutions of CEF and LZD

Accurately weighed quantities of CEF (10 mg) and LZD (10 mg) transferred to separate volumetric flasks (100 ml), dissolved in methanol (small quantity) and diluted up to mark with methanol (100 µg/ml of CEF and LZD).

Methodology

Method A: Simultaneous Equation Method

In quantitative estimation of two components by Simultaneous Equation method, two wavelengths i.e.277 nm of CEF and 257nm of LZD were selected as their respective λmax from the overlain spectrum (Figure 3-6) at which both drugs have absorbance. For simultaneous equation method, working standard solutions having concentrations 3, 5, 7, 9 and 11 µg/ml for CEF and 3.6, 6, 8.4, 10.8 and 13.2 µg/ml for LZD were prepared in methanol using the stock solutions. A set of two simultaneous equations were formed using absorptivity coefficients at selected wavelengths. The concentrations of two drugs in the mixture were calculated using the following two simultaneous equations. Statistical parameters like the slope, intercept coefficient correlation, standard deviation and relative standard deviation were calculated.

$Cx = A\,2\,ay\,1 - A\,1\,ay\,2 / ax\,2\,ay\,1 - ax\,1\,ay\,2$ …(1)

$Cy = A\,1\,ax\,2 - A\,2\,ax\,1 / ay\,1\,ax\,2 - ay\,2\,ax\,1$…(2)

where A_1 and A_2 are absorbances of the mixture at λ_1 and λ_2, respectively, ax_1 and ax_2 are absorptivity of X at λ_1 and λ_2, respectively, $ay_1$and ay_2 denote absorptivity of Y at λ_1 and λ_2, respectively, and Cx and Cy are concentrations of CEF and LZD, respectively.

Method B: Q-absorbance ratio method

In the quantitative assay of two components by Q-absorbance ratio method, absorbances were measured at two wavelengths, one being the isobestic wavelength and the other being wavelength of maximum absorption of one of the two components. From overlain spectra of CEF and LZD, absorbances were measured at the selected wavelength, i.e., 272 nm (isoabsorptive point) and 257 nm (wavelength of maximum absorption of LZD) (Figure 3). Working standard solutions having concentration concentrations 3, 5, 7, 9 and 11 µg/ml for CEF and 3.6, 6, 8.4, 10.8 and 13.2 µg/ml for LZD were prepared in methanol using stock solutions and the absorbances at 272 nm (isoabsorptive point) and 257 nm, (λ-max of LZD) were measured and the concentration of each component can be calculated by mathematical treatment of the following mentioned equation.

For CEF,

$C\,1 = Qm - Qy / Qx - Qy.\ A\,1 / a$ …(3)

For LZD,

$C\,2 = Qm - Qx / Qy - Qx.\ A\,1 / a$…(4)

where, C 1 = concentration of CEF

C 2 = concentration of LZD

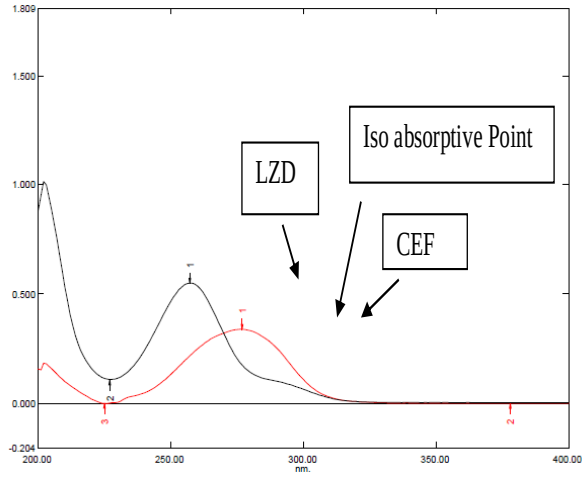

Figure 3: Overlain spectra of CEF and LZD

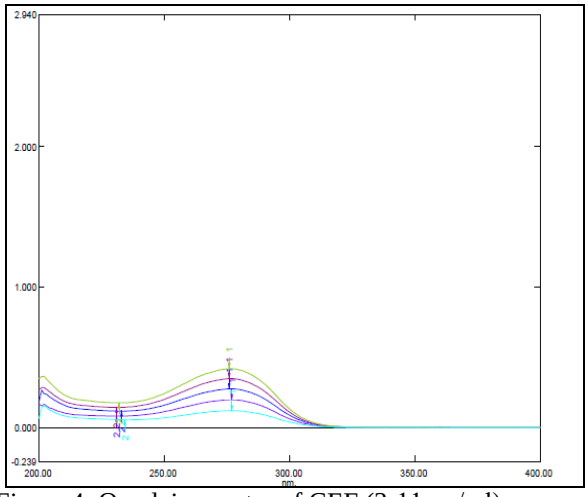

Figure 4: Overlain spectra of CEF (3-11 µg/ml)

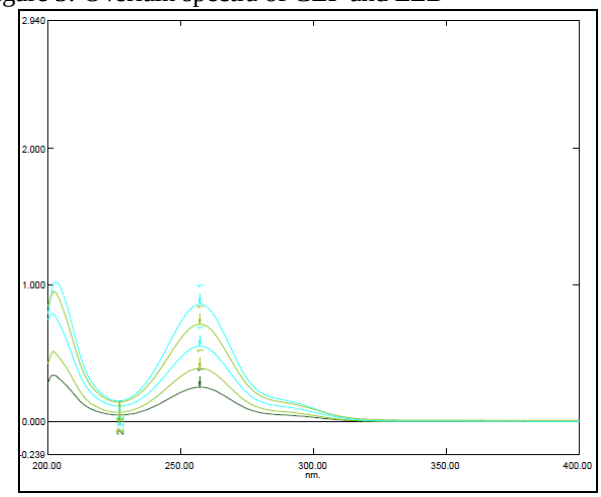

Figure 5: Overlain spectra of LZD (3.6-13.2 µg/ml)

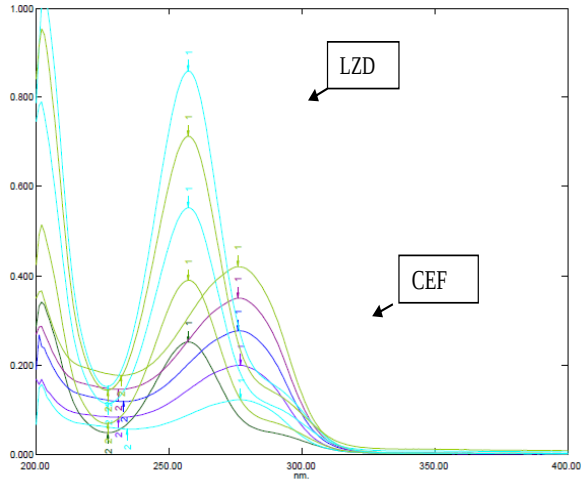

Figure 6: Overlain Spectra for CEF (3-11 µg/ml) & LZD (3.6-13.2 µg/ml)

A 1 = absorbance of sample at isoabsorptive wavelength (272 nm)

a = absorptivity of CEF and LZD at isoabsorptive wavelength (272 nm)

Qx = absorptivity of CEF at 257 nm/absorptivity of CEF at 272 nm

Qy = absorptivity of LZD at 257 nm/absorptivity of LZD at 272 nm

Qm = absorptivity of sample solution at 257 nm/absorptivity of sample solution at 272 nm.

Method C: First Derivative Spectroscopy Method

For first derivative spectrophotometric method, accurate aliquots of CEF equivalent to 10-30 µg/ml were transferred from its stock solution (100 µg/ml) into a series of 10 ml volumetric flasks and diluted to mark with methanol and mixed well. Accurate aliquots of LZD equivalent to 12-36 µg/ml were transferred from its working solution (100 µg/ml) into a series of 10 ml volumetric flasks and diluted to mark with methanol and mixed well. Considering all the derivative order spectra of CEF and LZD from first to fourth derivative, the first derivative order spectra with d (N) =2 was found suitable. The zero crossing point on the first derivative spectra of one drug, the other drug shows substantial absorbance, these two wavelengths can be employed for the estimation of CEF and LZD without any interference from other drug in combined formulations. From the derivatised spectra of prepared mixtures the absorbances were measured at 256 nm for CEF and 275 nm for LZD. These absorbances Vs concentration were plotted in the quantitative mode to obtain the working curves from which by extrapolating the value of absorbances of the sample solution, the concentration of the corresponding drugs were determined. Both the drugs obeyed Beer's Law.

Analysis of CEF and LZD in tablets

Marketed tablets formulations containing CEF (500 mg) and LZD (600 mg) were analyzed using these three methods. From the triturate of 20 tablets, an equivalent to 10 mg of CEF and 12 mg of LZD was weighed and dissolved in 10 ml of methanol in 100 ml volumetric flask by sonication for 15 mins. Then final volume of the solution was made upto 100 ml with methanol to get final concentration of 100 μg/mL of CEF and 120 μg/mL of LZD. The solution was filtered through whatmann filter paper no.41 and filtrate was appropriately diluted to get approximate concentration of 5 μg/mL of CEF and 6 μg/mL of LZD for method A & B and 10 μg/mL of CEF and 12 μg/mL of LZD for method C. Absorbances of sample solutions were recorded at 277 nm and 257 nm

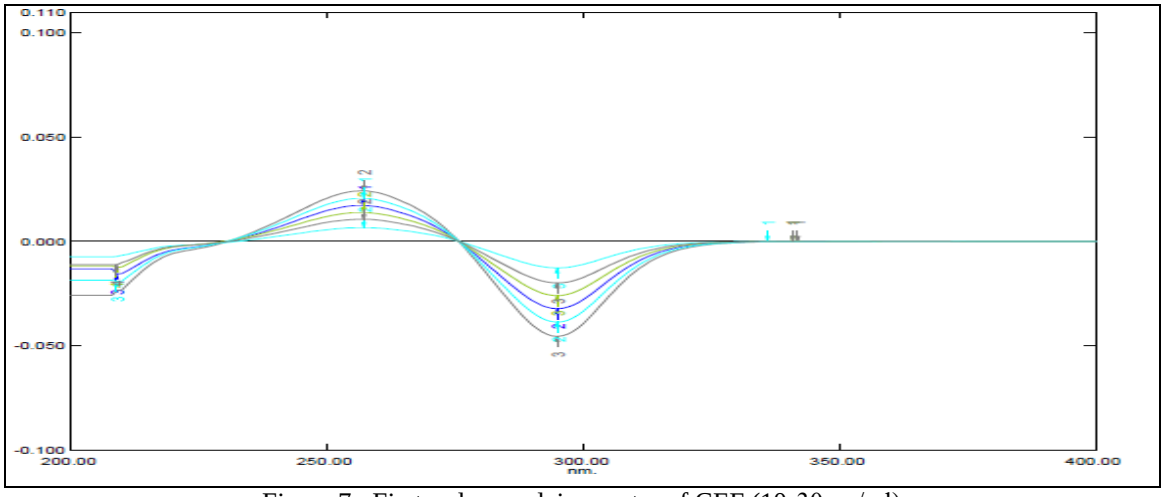

Figure 7 : First order overlain spectra of CEF (10-30 µg/ml)

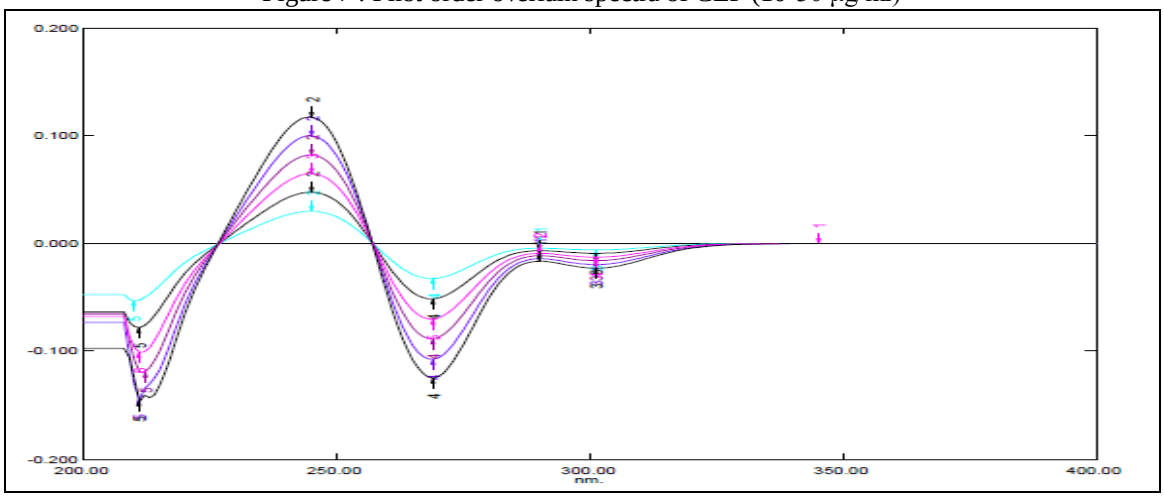

Figure 8: First order overlain spectra of LZD (12-36 µg/ml)

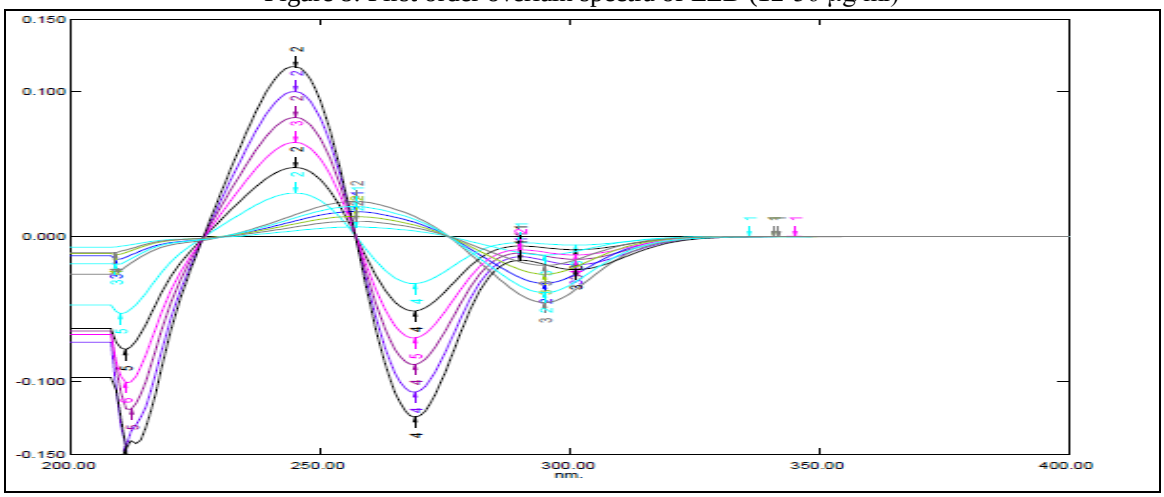

Figure 9: First order overlain Spectra for CEF (10-30 µg/ml) & LZD (12-36 µg/ml)

and the concentration of two drugs in the sample were determined by using eqns.1 and 2 (Method-A). Absorbances of sample solutions were recorded at 272 nm (Isoabsorptive Point) and 257 nm (λ max of LZD) and concentration of two drugs in the sample were determined by using equations 3 and 4 (Method B). For First derivative method (Method C) the absorbance was measured at 256 nm (ZCP of LZD) and 275 nm (ZCP of CEF). The concentration of each analyte was determined with the equations generated from calibration curve of respective drugs.

Validation parameters

Validation was carried out according to ICH guideline[23].

Accuracy

For studying the accuracy of the proposed methods, and for checking the interference from excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. This study was performed

Table 4: One way ANOVA for CEF

Source of Variation	Sum of Square	Degree of freedom	Mean Squares	F_{cal}	P-value	F_{tab}
Between Groups	1.12	2	0.56	2.763158	0.095138	3.68232
Within Groups	3.04	15	0.202667			
Total	4.16	17				

Table 5: One way ANOVA for LZD

Source of Variation	Sum of Square	Degree of freedom	Mean Squares	F_{cal}	P-value	F_{tab}
Between Groups	0.426178	2	0.213089	1.160397	0.339959	3.68232
Within Groups	2.754517	15	0.183634			
Total	3.180694	17				

by addition of known amounts of CEF and LZD to a known concentration of sample solution. The amounts of standard recovered were calculated in terms of mean recovery with the upper and lower limits of % R.S.D.

Precision

Repeatability

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n = 6) for CEF and LZD without changing the parameter of the proposed spectrophotometry methods.

Intermediate Precicion

Intra-day precision and inter-day precision for the developed methods were measured in terms of % R.S.D. The experiments were repeated three times a day for intra-day precision and on 3 different days for inter-day precision. The concentration values for both intra-day precision and inter-day precision were calculated three times separately and % R.S.D. were calculated.

Limit of detection (LOD) and limit of quantitation (LOQ)

Limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to the 3s/m and 10s/m criterions, respectively, where s is the standard deviation of intercept (n =6) of the sample and m is the slope of the corresponding calibration curve.

ANOVA

Statistical analysis was performed to assess the effect of three methods in simultaneous estimation of CEF and LZD using one-way analysis of variance ($P < 0.05$)[24].

RESULTS AND DISCUSSION

Method A: Simultaneous Equation Method

Figure 3-6 explains overlain spectra for CEF and LZD which absorb at each other's λ max. Analytical wavelengths CEF and LZD were 277 nm and 257 nm selected respectively. So equations 1 and 2 were directly utilized for the simultaneous estimation of CEF and LZD in sample solution.

Method B: Q-absorbance ratio method

In Q-absorbance ratio method, the primary requirement for developing a method for analysis is that the entire spectra should follow the Beer's law at all the wavelength, which was fulfilled in case of both these drugs. The two wavelengths were used for the analysis of the drugs were 272 nm (isoabsorptive point) and 257 nm (λ-max of LZD) at which the calibration curves were prepared for both the drugs.

Method C: First Derivative Spectroscopy Method

In contrast to zero-order spectra, first derivative spectra show more resolution in terms of zero crossing points shown in Figure 7-9 explains first order derivative spectra for CEF and LZD. At 256 nm, CEF having zero crossing point and LZD can be determined. At 275 nm, CEF having zero crossing point and LZD can be determined. Table 1, Table 2 and Table 3 exhibits results of assay, results of accuracy studies and summary of various validation parameters of all methods respectively.

Statistical comparison of the results of the developed three methods

Method A, B and C were compared using one-way ANOVA[24] and no significant difference was found between them as the F_{cal} value is less than F_{tab}. The results of one-way ANOVA are shown in table 4 and 5.

CONCLUSION

Three spectrophotometric methods (Simultaneous equation method, Q-absorption ratio method and first derivative spectrophotometry) were developed for simultaneous estimation of CEF and LZD in their combined pharmaceutical formulation without prior separation. Methods were found to be precise and accurate as can be reflected from validation data. Developed methods were successfully applied for estimation of CEF and LZD in formulation. The one-way ANOVA results show that there is no significant difference between assay results obtained from these three methods. So the proposed methods can be used in routine analysis of CEF and LZD with relatively less expensive and simple to operate instrumentation.

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