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Research

Advanced Method Development And Validation For Analyzing Cefixime Trihydrate And Levofloxacin Hemihydrate In Tablet Formulations Using First-Order Derivatives

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Abstract

The research paper is concerned with the Analyzing Cefixime Trihydrate and Levofloxacin Hemihydrate in Tablet Formulations Using First-Order Derivatives presents a novel approach to pharmaceutical analysis through derivative spectrophotometry. This method involves transforming standard absorption spectra into first-order derivative spectra, enhancing the precision and resolution of substance identification. By analyzing the rate of absorbance change with wavelength(CEFI 297.2 nm & LEVO 288.6 nm), specific spectral features can be pinpointed, facilitating accurate quantification and characterization of substances. Utilizing a UV-visible spectrophotometer with advanced software, the study focused on the analysis of Cefixime Trihydrate and Levofloxacin Hemihydrate, common pharmaceutical compounds. The validation process adhered to international guidelines, ensuring the method's reliability and accuracy (CEFI 100.4 ± 0.94 & LEVO 100.4 ± 0.82). Calibration curves exhibited linearity over a defined concentration range for both compounds, validating the method's suitability for quantitative analysis. Overall, this study contributes to the advancement of analytical techniques for pharmaceutical quality control and research, emphasizing the pivotal role of derivative spectrophotometry in modern analytical practices.

Keywords: Cefixime , Levofloxacine, Spectrophotometric, First order Dervivative.

INTRODUCTION

Derivative spectrophotometry involves spectral analysis in order to relate chemical structure to electronic transitions, and for analytical situations in which mixture contribute interfering absorption, a method of manipulating the spectral data is called derivative spectroscopy[(1)]. Derivative spectrophotometry involves conversion of a normal spectrum to its first, second and higher derivative spectrum. In the context of derivative

spectrophotometry, the normal absorption spectrum is referred to as the fundamental, zero order, or D^0 spectrum. The first derivative D^1 spectrum is a plot of the rate of change of absorbance with wavelength against wavelength i.e. a plot of the slope of the fundamental spectrum against wavelength or a plot of dA/dλ vs λ .The maximum positive and maximum negative slope respectively in the D spectrum correspond with a maximum and minimum respectively in the D^1 spectrum[2] [3]. The max in D spectrum is a wavelength of zero slope and gives dA/dλ = 0 in the D^1 spectrum. The second derivative D2 spectrum is a plot of the curvature of the D spectrum against wavelength or a plot of d2A/dλ2 vs λ. The maximum negative curvature in the D spectrum gives two small maxima called satellite bands in the D2 spectrum, and the maximum positive curvature in the D spectrum gives two small maxima called satellite bands in the D2 spectrum. The wavelength of maximum slope and zero curvature in the D spectrum correspond with cross-over points in the D2 spectrum. These spectral transformations confer two principal advantages on derivative spectrophotometry[4] [6]. Firstly, Zero order spectrum is of narrower spectral bandwidth than its fundamental spectrum. A derivative spectrum therefore shows better resolution of overlapping bands than the fundamental spectrum and may permit the accurate determination of the max of the individual bands. Secondly, derivative spectrophotometry discriminates in favour of substances of narrow spectral bandwidth against broad bandwidth substances[6]. All the amplitudes in the derivative spectrum are proportional to the concentration of the analyte, provided that Beer's law is obeyed by the fundamental spectrum. The enhanced resolution and bandwidth discrimination increases with increasing derivative order. However, it is also found that the concomitant increase in electronic noise inherent in the generation of the higher order spectra, and the consequent reduction of the signal to noise ratio, place serious practical limitations on the higher order spectra. For quantitative purposes, second and fourth derivative spectra are the most frequently employed derivative orders[8].

A first-order derivative is the rate of change of absorbance with respect to wavelength. A first order derivative starts and finishes at zero. It also passes through zero at the same wavelength as _max of the absorbance band[2]. Either sides of this point are positive and negative bands with maximum and minimum at the same wavelengths as the inflection points in the absorbance band. This bipolar function is characteristic of all odd-order derivatives. Derivative spectra may be generated by any of three techniques[12]. The earliest derivative spectra were obtained by modification of the optical system. Spectrophotometers with dual monochromator set a small wavelength interval (Δλ, typically 1-3 nm) apart or with the facility to oscillate the wavelength over a small range, are required. In either case the photo detector generates a signal with amplitude proportional to the slope of the spectrum over the wavelength interval. Instruments of this type are expensive and are essentially restricted to the recording of first derivative spectra only. The second technique to generate derivative spectra is electronic differentiation of the spectrophotometer analog signal[23]. Resistance Capacitance modules may be incorporated in series between the spectrophotometer and recorder to provide differentiation of the absorbance, not with respect to wavelength, but with respect to time, thereby producing the signal dA/dt. If the wavelength scan rate is constant (dλ/dt = Ce), the derivative with respect to wavelength is given by,

$$DA/d = (dA/dt)/ (d\lambda/dt) = (dA/dt) (1/C)$$

Derivative spectra obtained by RC method are highly dependent on instrumental parameters, in particular the scan speed and the time constant. It is essential, therefore, to employ a standard solution of the analyte to calibrate the measured value the instrumental conditions selected. The third technique is based upon microcomputer differentiation. Microcomputers incorporated into or interfaced with the spectrophotometer may be programmed to provide derivative spectra during or after the scan, to measure derivative amplitudes between specified wavelengths and to calculate concentrations and associated statistics from the measured amplitude[30].

Experimental

Apparatus

A double beam UV-visible spectrophotometer (Shimadzu, UV-1700, Japan), attached to a computer software UV probe 2.0, with a spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells.Analytical balance (CP224S, Sartorius, Germany)Ultrasonic cleaner (Frontline FS 4, Mumbai, India).Corning volumetric flasks and pipettes of borosilicate glass were used in the study.

Reagents and materials

Cefixime Trihydrate (CEFI) and Levofloxacin Hemihydrate (LEVO) were kindly supplied as a gift samples from Acme Pharmaceuticals, Kherva, Mehsana, Gujarat, India.AR grade methanol (S.D. Fine Chemical Ltd., Mumbai, India.).Whatman filter paper no. 41 (Whatman International Ltd., England).

Preparation of solutions

Preparation of standard stock solution

Accurately weighed portion of CEFI (10 mg) and LEVO (10 mg) was transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with methanol to obtain standard solution having concentration 100 µg/ml .

Preparation of marketed formulated solution

Twenty tablets were taken, crushed and the powder was weighed. The equivalent weight was taken; the powder with equivalent weight was transferred to 100 ml volumetric flask and diluted to the mark with methanol. Then pipette out (0.2 ml) of the above solution and was transferred to 10 ml volumetric flask. Then pipette out 1.0 ml of the above solution and was transferred to 10 ml volumetric flask. The volume was adjusted with methanol. Final mixture was prepared CEFI (8 µg/ml) and LEVO (10 µg/ml).

Method Development

Determination of the zero crossing points

The standard solutions of CEFI (10 µ g/ml) and LEVO (10 µg/ml) were scanned separately in the UV range of 200-400 nm. The zero order spectra thus obtained was then processed to obtain first derivative spectrum. At ZCP of first drug, second drug showed reasonable absorbance, while at ZCP of second drug, first drug showed reasonable absorbance so these two wavelengths were selected for further measurement.

Preparation of calibration curve

The calibration curves were plotted over a concentration range of 2-24 µg/ml for CEFI and 2-14 µg/ml for LEVO. Accurately measured mixture solutions of CEFI (0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4 ml) and of LEVO (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with methanol. The absorbance of derivatised spectra was measured at 297.2 nm (zero crossing point for CEFI) and 288.6 nm (zero crossing point for LEVO) against methanol as blank.

Method validation

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines.

Linearity

Linearity was observed in a concentration range of 2-24 µg/ml for CEFI and 2-14 µg/ml LEVO. The calibration curve was constructed by plotting the graph of absorbance Vs concentration.

Range

Range is the interval between upper and lower concentration of analyte for which it has been demonstrated that the analytical method has suitable level of precision, accuracy and linearity. The range for the method was observed in a concentration range of 2-24 µg/ ml for CEFI and 2-14 µg/ml for LEVO. For the evaluation of the range accurately measured mixture standard working solutions of CEFI (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 ml) and LEVO (0.2, 0.4, 0.8, 1.2, 1.6 2.0, 2.4 ml) were pipette out in to a separate series of 10 ml volumetric flasks. The volume was adjusted with methanol and absorbencies of derivatised spectra were measured at 297.2 nm (zero crossing point for CEFI) and 288.6 nm (zero crossing point for LEVO) against methanol as blank absorbance.

Method precision (Repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n = 6) for CEFI and LEVO (10 µg/ml for both drugs) wit hout changing the parameter of the proposed first order derivative method. The results are reported in terms of relative standard deviation (% RSD).

Intermediate precision (Reproducibility)

The intraday and interday precision of the proposed first order derivative method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of CEFI and LEVO (2, 4, 8 µg/ml for CEFI and 2, 4, 8 µg/ml for LEVO). The result was reported in terms of relative standard deviation (% RSD).

Limit of detection and limit of quantification

LOD and LOQ were calculated from the data obtained from the linearity studies. The slope of the linearity plot was determined. For each of the six replicate determinations, y intercept was calculated and the

standard deviation of the y intercept was computed. From these values, the limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where,

σ = the standard deviation of the response and S = slope of the calibration curve

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recovery of CEFI and LEVO by the standard addition method. Known amounts of standard solutions of CEFI and LEVO were added at 80, 100 and 120 % level to prequantified sample solutions of CEFI and LEVO (0.5 µg/ ml for CEFI and LEVO). The solutions were measured at 297.2 nm for CEFI and 288.6 nm for LEVO and % recovery of the sample were calculated.

Analysis of drugs in tablet formulation

From the Tablet formulation CEFI-L (400 mg CEFIXIME and 500 mg LEVOLOXACIN) one tablet was crushed powdered and was transferred in 100 ml volumetric flask. 0.2 ml taken in 10 ml volumetric flask and the volume was adjusted to mark with methanol. This solution (1 ml) was taken in to a 10 ml volumetric flask and the volume was adjusted up to mark with methanol to get a final concentration of CEFI (8 µg/ml) and LEVO (10 µg/ml) and their first derivative spectra were recorded. From the derivative spectra, the absorbance at 297.2 nm and 288.6 nm were noted for the estimation of CEFI and LEVO, respectively. From these absorbance values, the concentrations of CEFI and LEVO were determined using calibration graph. The analysis procedure was repeated six times with the tablet formulation.

RESULTS AND DISCUSSION

Method Development

The working standard solution of CEFI and LEVO were prepared separately in methanol. They were scanned in the wavelength range of 200-400 nm. From the overlay derivatised spectra of two drugs, it is evident that CEFI and LEVO show a zero crossing point at 297.2 nm and 288.6 nm. These two wavelengths were employed for the determination of CEFI and LEVO. Overlain and derivatised spectra of both the drugs are shown in Figure 1 & 2.

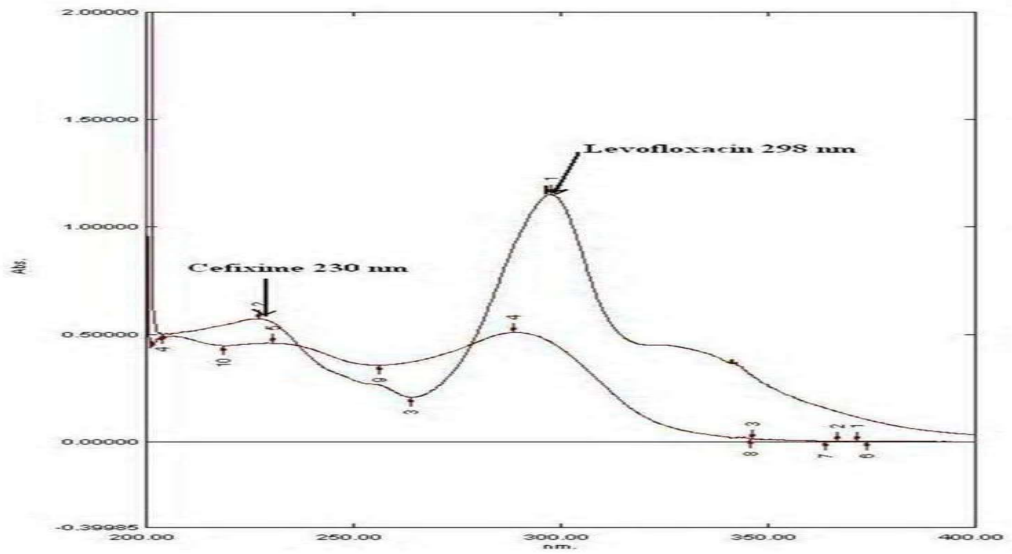

Fig 1 : Overlain UV zero order absorption spectra of CEFI and LEVO in methanol

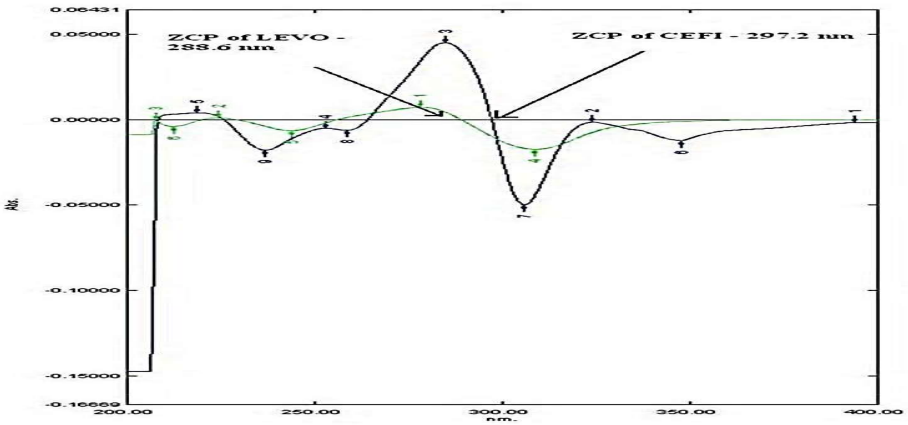

Fig 2: Overlain UV first order absorption spectra of CEFI and LEVO in methanol

Validation of the derivative spectroscopy method

Linearity

Calibration range was observed in the concentration range of 2-24 µg /ml for CEFI and 2-14µg/ml for LEVO both. The calibration curves at different wavelengths are shown in Figure. 3, 4.

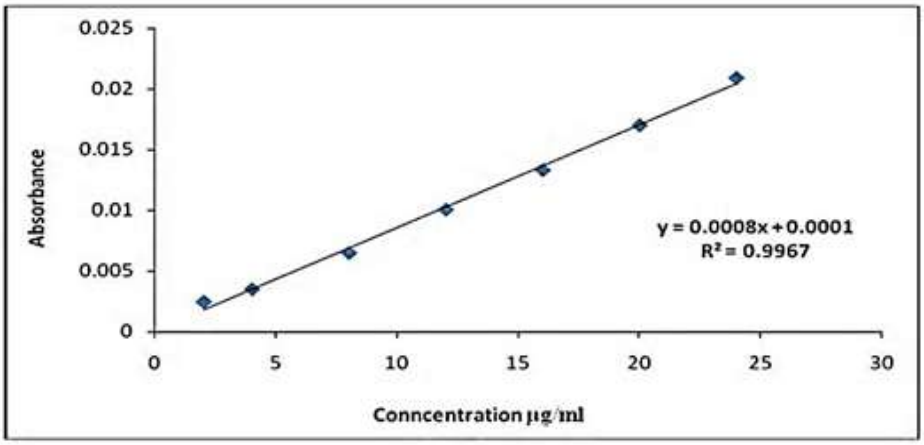

Fig 3: Calibration Curve of CEFI at 297.2 nm

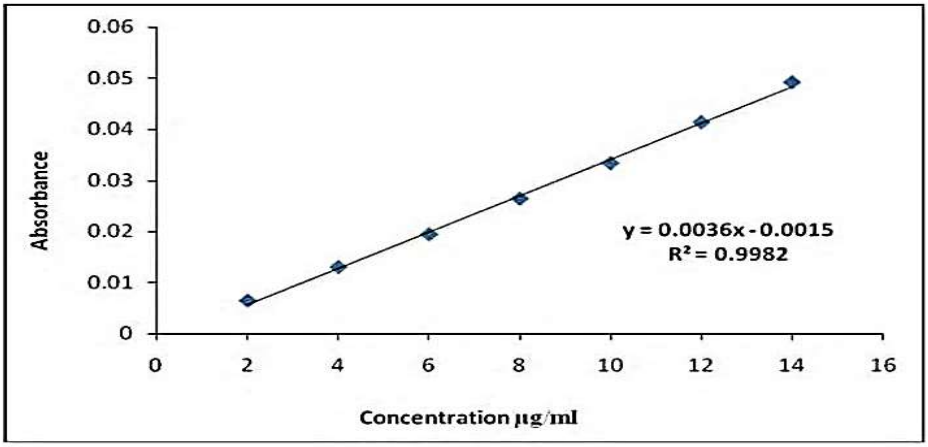

Fig 4: Calibration Curve of LEVO at 288.6 nm

Table 1: Regression Analysis Data and Summary of Validation Parameters for the First derivative Method

PARAMETERS		CEFI	LEVO
Wavelength (nm)		297.2	288.6
Beers law limit (µg /ml)		2-24	2-14
Regression equation Y=mX + c		Y = 0.0008 x ± 0.0001	Y = 0.0036 x ± 0.0015
Slope		0.0008	0.0036
Intercept		0.0001	0.0015
Correlation coefficient (r^2)		0.9967	0.9982
Repeatability (%RSD , n =3)		1.06	0.36
Precision (%RSD)	Intraday (%RSD)	0.53– 1.02	0.64 -0.96
	Interday (%RSD)	0.47 -0.9366	0.7711 -1.1282
LOD (µg /ml)		0.3747	0.2750
LOQ (µg /ml)		1.13	0.83
(Accuracy ± S.D) % Recovery n= 3		100.4 ± 0.94	100.4 ± 0.82

Method Precision (Repeatability)

The RSD values of CEFI and LEVO were found to be 1.06 and 0.36 % respectively at 297.2 nm & 288.6 nm respectively (Table 2). Low value of RSD indicates that proposed method is repeatable.

Table 2: Repeatability data of proposed Method (n=6)

Concentration (CEF : LEVO) (12 : 12 µg /ml)	CEFIXIME	LEVOFLOXACIN
Wavelength (nm)	297.2 nm	288.6nm
1	0.0103	0.0412
2	0.0102	0.0413
3	0.0102	0.0415
4	0.0104	0.0415
5	0.0105	0.0412
6	0.0103	0.0415
Mean	0.0103	0.0413
S.D	0.00011	0.00015
Repeatability (%RSD , n =6)	1.06	0.36

Intermediate Precision (Reproducibility)

The RSD values of CEFI for interday (0.53-1.02 %) and intraday (0.47 – 0.93 %) at 297.2 nm and the RSD values of LEVO for interday (0.64 – 0.96 %) and intraday (0.77-1.12 %) at 288.6 nm.

LOD and LOQ

LOD and LOQ values for CEFI found to be 0.37 and 1.13 µg/ml at 297.2 nm, and LEVO were found to be 0.27 and 0.83µg /ml at 288.6 nm. Low value of LOD & LOQ indicates that the method is sensitive. (Table1).

Accuracy (% Recovery)

The recovery experiments were performed by the standard addition method. The mean recoveries were found to be 100.4± 0.94 and 100.4± 0.82 for CEFI and LEVO, respectively. The recoveries results indicate that the proposed method is accurate. Results of recovery studies are shown in Table 3.

Table 3: Recovery data of proposed method

Drug	Level	Amount taken (µg /ml)	Amount added (%)	Mean recovery ± S.D (n= 3)
CEFI	I	8.0	80	100.7 ± 0.95
	II	8.0	100	99.8 ± 1.00

	III	8.0	120	100.2 ± 0.88
LEVO	I	10.0	50	101.5 ± 0.72
	II	10.0	100	99.6 ± 0.48
	III	10.0	150	100.1 ± 1.27

S.D is Standard deviation and n is number of replicate

Assay

The proposed validated method was successfully applied to determine CEFI and LEVO in tablet formulation. Results are given in Table 6.4. No interference of the excipients with the absorbance of analyte of interest appeared; hence the proposed method is suitable for the routine analysis of CEFI and LEVO in combined dosage forms.

Table 4: Analysis of CEFI and LEVO in Tablet formulation by Derivative Spectrophotometric method (n=6)

Tablet Formulation	Label claim (mg)		Amount find (mg)		% Label claim (mg) (n=6)	
	CEFI	LEVO	CEFI	LEVO	CEFI	LEVO
1	400	500	406.62	509	100.75	101.86
2	400	500	406.58	507	101.66	101.13
3	400	500	406.57	501.09	101.64	101.39
4	400	500	405.9	497.4	100.42	99.49
5	400	500	399	496.5	99.80	99.32
6	400	500	401	484	100.19	98.81
Mean			403.7	499.16	100.5	100.58
S.D					0.63	1.16

Table 5: Summary of validation parameters for First derivative spectrophotometric method

First derivative spectrophotometric method.										
Parameters	**Wavelength Range (nm)**	**Range (µg /ml)**	**Correlation Coefficient (r^2)**	**Mean Recovery (n=3)**	**Repeatability (RSD% n=6)**	**Inter – day (RSD% n=3)**	**Intra-Day (RSD%n=3)**	**LOD**	**LOQ**	**Assay (n=6)**
CEFI	297.2	2-24	0.9986	99.75 ±1.25	0.02	0.53-1.02	0.47-0.93	0.37	1.13	100.4±0.94
LEVO	288.62	2-14	0.9987	100.1±1.17	0.05	0.64-0.96	0.77-1.13	0.27	0.83	100.4± 0.8

CONCLUSION

Based on the results obtained from the analysis using proposed method, it can be concluded that the method has linear response in the range of 2-24 µg/ml and 2-14 µg/ml for CEFI and LEVO, respectively. The result of the analysis of tablet formulation by the proposed method is highly reproducible and reliable and is in good agreement with label claim of the drugs. The additive present in the tablet formulation did not interfere in the analysis. So the method can be used for the routine analysis of drugs in combined dosage form.

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