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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHODS FOR THE SIMULTANEOUS ESTIMATION OF LEVOFLOXACIN AND PHENYLPROPANOLAMINE IN BULK AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

UV visible spectrophotometric method is very frequently employed in pharmaceutical analysis. It involves the measurement of the amount of ultraviolet (200-400nm) or visible (400-800nm) radiation absorbed by a substance in solution by an instrument which measures the ratio or a function of the ratio of the intensity of two beams of light in UV-Visible region. The basis of all spectrophotometric methods for multicomponent sample analysis is the property that the absorbance of a solution is the sum of absorbances of individual components or the measured absorbance is the difference between total absorbance of the solution in the sample cell and that of the solution in the reference (blank) cell.

KEY WORDS

UV-Visible spectrophotomentric methods, Absorbances

INTRODUCTION:

UV visible spectrophotometric method is very frequently employed in pharmaceutical analysis. It involves the measurement of the amount of ultraviolet (200-400nm) or visible (400-800nm) radiation absorbed by a substance in solution by an instrument which measures the ratio or a function of the ratio of the intensity of two beams of light in UV-Visible region. The basis of all spectrophotometric methods for multicomponent sample analysis is the property that the absorbance of a solution is the sum of absorbances of individual components or the measured absorbance is the difference between total absorbance of the solution in the sample cell and that of the solution in the reference (blank) cell. The various spectrophotometric methods which are used for estimation of drug in combined dosage form include simultaneous equation method, absorbance ratio method, derivative spectrophotometry and dual wavelength method.

Levofloxacin (Fig.:1) is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. Levofloxacin acts as a bactericide. Chemically Levofloxacin is (-) -(S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido [1, 2, 3-de] - 1, 4-benzoxazine-6-carboxylic acid hemihydrate.

Phenylpropanolamine (Fig.:2) is a sympathomimetic agent structurally similar to pseudoephedrine, is used to treat nasal congestion. Phenylpropanolamine is found in appetite suppressant formulations and with guaifenesin in cough-cold formulations. Phenylpropanolamine acts directly on alpha- and, to a lesser degree, beta-adrenergic receptors in the mucosa of the respiratory tract. Stimulation of alpha-adrenergic receptors produces vasoconstriction, reduces tissue hyperemia, edema, and nasal congestion, and increases nasal airway potency. Chemically Phenylpropanolamine is (1S, 2R)-2-amino-1-phenylpropan-1-ol.

The combination of Levofloxacin and Phenylpropanolamine is prescribed for treating certain bacterial infections and preventing anthrax. It is quinolone antibiotic. It kills sensitive bacteria.

On literature survey, Levofloxacin alone has been estimated and simultaneous estimation in combination with other drugs has been reported. Phenylpropanolamine alone has been estimated and simultaneous estimation in combination with other drugs has been reported. It was found that no method has been reported for the simultaneous estimation of Levofloxacin and Phenylpropanolamine in combined dosage form and no method is available in the pharmacopoeias. In the view of the need for a suitable method for routine analysis in combined formulations, attempts are being made to develop simple, precise and accurate analytical methods for simultaneous estimation of titled drugs and extend it for their determination in formulations.

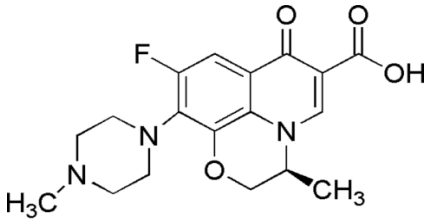


Fig 1: Chemical structure of Levofloxacin.

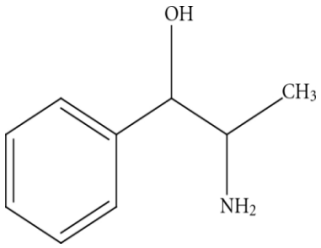


Fig 2: Chemical structure of Phenylpropanolamine.

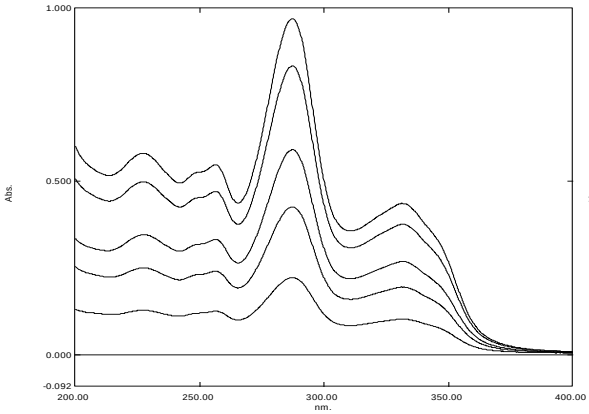


Fig. 3: Overlay Spectrum of LEVO at 287nm.

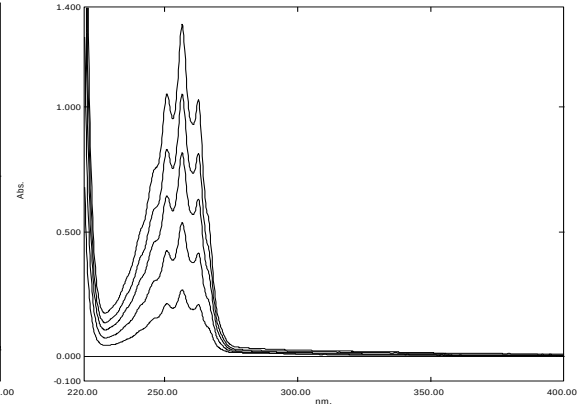


Fig. 4: Overlay Spectrum of PPA at 256nm.

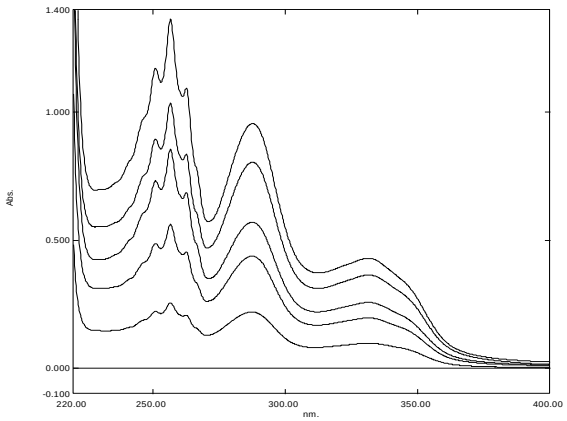


Fig. 5: Overlay Spectrum of Standard Mixture.

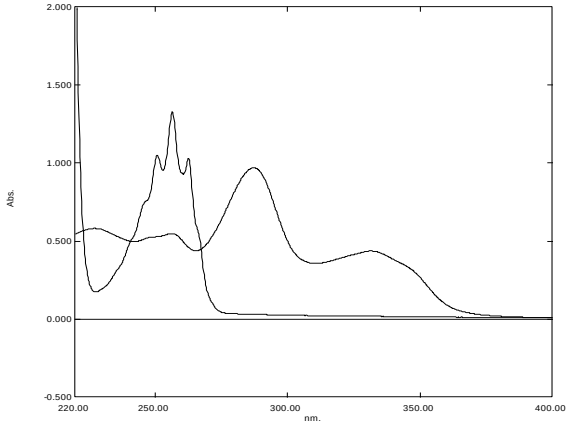
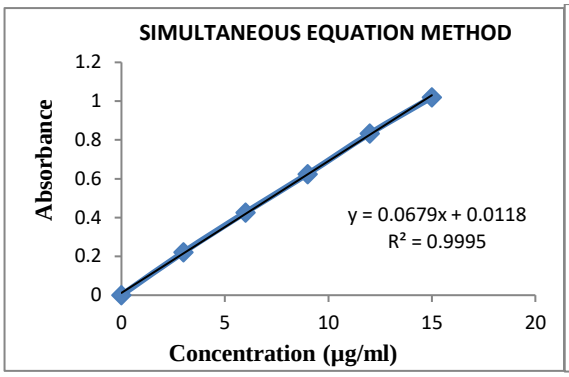


Fig. 6: Overlay Spectrum of LEVO and PPA at 287nm and 256nm.



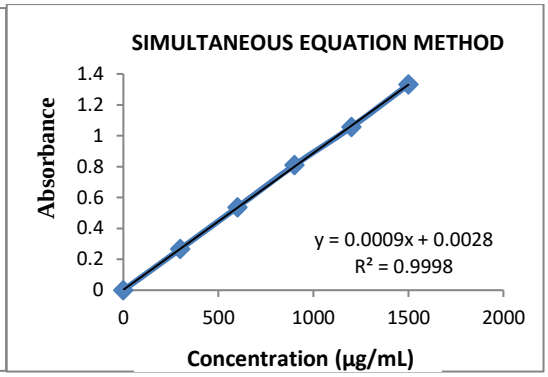


Fig. 7: Calibration Curve for LEVO at 287nm for Simultaneous Equation Method.

Fig. 8: Calibration curve for PPA at 256nm for Simultaneous Equation Method.

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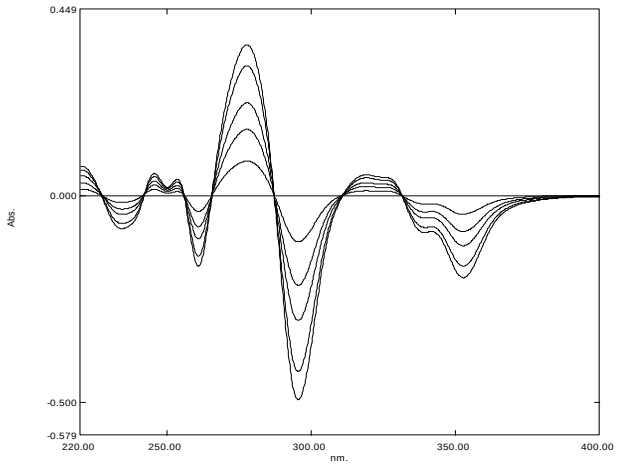


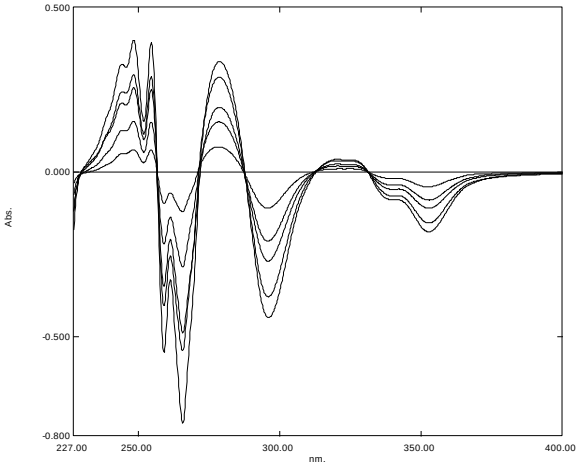
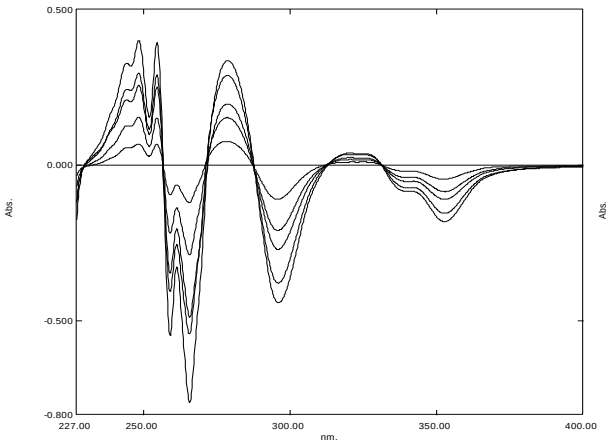


Fig. 9: Overlay of First order Derivative Spectrum of LEVO at 295nm.

Fig. 10: Overlay of First order Derivative Spectrum of PPA at 248nm.



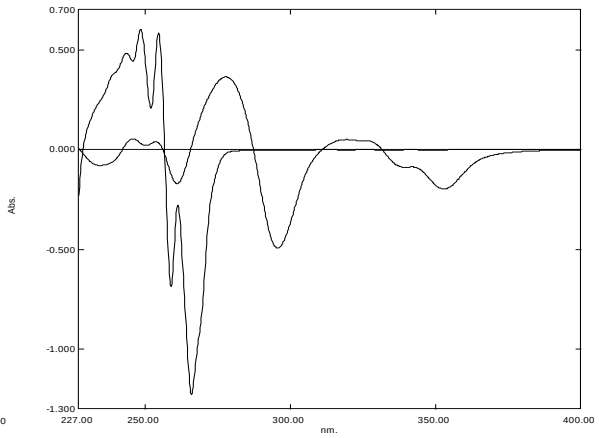
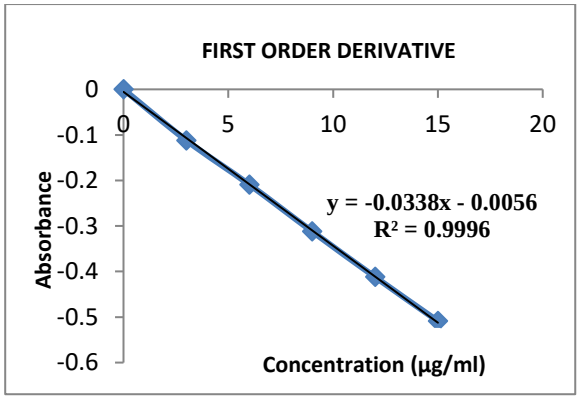


Fig. 11: Overlay of First order Derivative Spectrum of Standard mixture.

Fig. 12: Overlay of First order Derivative Spectrum of LEVO & PPA at 295nm & 248nm



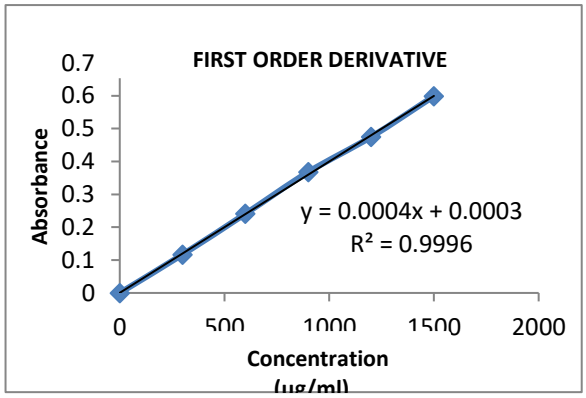


Fig. 13: Calibration Curve for LEVO at 295nm by First Order Derivative.
Fig. 14: Calibration Curve for PPA at 248nm by First Order Derivative

MATRIALS AND METHODS:

Instrument

For UV-Visible Spectroscopy methods, Shimadzu model 1800 double beam UV-Visible Spectrophotometer with spectral band width of 1 ± 0.2nm, wavelength accuracy of ±0.3nm and a pair of quartz cuvettes having 1cm path length was used. Distilled water was used throughout the experimental work.

Chemicals

Standard Levofloxacin was obtained as gift sample from micro labs, Bangalore. Standard Phenylpropanolamine was procured from yarrow chem products, Mumbai.

Methods

Preparation of standard solutions

Preparation of standard solution of Levofloxacin (LEVO)

100mg of Levofloxacin was weighed and transferred to 100 ml volumetric flask. Drug was dissolved in 50 ml distilled water by ultra-sonication and volume was made upto the mark with distilled water to obtained finally concentration of 1000µg/ml (stock A). From the above stock A solution 10ml of aliquot was pipetted out 100 ml volumetric flask and volume was made upto the mark with the distilled water to obtain a concentration of 100µg/ml (stock B). From the above stock B solution further dilutions were made to get concentration range from 3-15µg/ml for Levofloxacin.

Preparation of standard solution of Phenylpropanolamine (PPA)

200mg of PPA was weighed and transferred to 100 ml volumetric flask. Drug was dissolved in 70 ml distilled water by ultra-sonication and volume was made upto the mark with distilled water to obtain a concentration of 2000µg/ml (stock A). From the above stock A solution further dilutions were made to get concentration range from 300-1500µg/ml for Phenylpropanolamine.

Preparation of sample solution

20 tablets which contains both LEVO and PPA were weighed and powdered. The tablet powder equivalent to one tablet was weighed accurately and dissolves in 70 ml distilled water and sonicated for 15mins. The solution was filtered through Whatmann filter paper No. 41, finally the volume was made up to the mark with distilled water. Further dilutions were made to bring the concentration of the drugs within the range.

Method of estimation

Method A (Simultaneous equation method)

From the above standard solution both drugs were prepared and scanned in the wavelength range of 400-200nm using UV – Spectrophotometer. At 287nm LEVO showed maximum absorbance and at 256nm PPA shows maximum absorbance. Both drugs did not show any interference at either of the wavelength. Hence 287nm and 256nm for LEVO and PPA were selected as the working analytical wavelength.

$C_x = A_1ay_2 – A_2ay_1 / ax_1ay_2 - ax_2ay_1$

$C_y = A_2ax_1 – A_1ax_2 / ax_1ay_2 – ax_2ay_1$

Where,

C_x = absorbance of Sample at 287nm

C_y = absorbance of Sample at 256nm

ax_1 = absorptivity of Levofloxacin at 287nm

ax_2 = absorptivity of Levofloxacin at 256nm

ay_1 = absorptivity of Phenylpropanolamine at 287nm

ay_2 = absorptivity of Phenylpropanolamine at 256nm

Method B (First order derivative)

For the estimation of Levofloxacin and Phenylpropanolamine by first order derivative spectroscopy, zero crossing point for both drugs were obtained, and the wavelengths were selected in manner

such that at the zero crossing of one drug, the other drug should show substantial absorbance. From the first order derivative spectra of standard Levofloxacin and Phenylpropanolamine, zero crossing point of Levofloxacin was found at 287nm and zero crossing point of Phenylpropanolamine was found at 256nm and wavelength selected for their estimation was 295nm for LEVO and 248nm for PPA.

VALIDATION PARAMETRE:

Linearity

In Method A (Fig. 3 to 6) overlay spectra of both drugs and their mixtures were shown. Fig.7 and Fig.8 were shown linearity of both the drugs in their respective wavelengths. The responses of simulations equation for both drugs show linear concentration range of 3-15µg/ml and 300-1500µg/ml for LEVO and PPA respectively. The regression equation calculated by least square method was y =0.0679x + 0.0118 and y = 0.0009x + 0.0028 with correlation coefficient of both drugs was r^2 = 0.9995 and r^2 = 0.9998.

In Method B (Fig. 9 to 12) overlay spectra of both drugs and their mixtures were shown. Fig.13 and Fig 14 were shown linearity of both the drugs in their respective wavelengths. The responses of first derivatives both drugs show linear concentration range of 3-15µg/ml and 300-1500µg/ml for LEVO and PPA respectively. The regression equation calculated by least square method was y = -0.00338x – 0.0056 and y = 0.0004x + 0.0003 with correlation coefficient of both drugs was r^2= 0.9996 and r^2 = 0.9996. Summary of validation parameters by developed methods as shown in Table no 1.

Table 1: Summary of Validation Parameters by Developed Methods.

Parameter	Method A		Method B	
	LEVO	PPA	LEVO	PPA
Wavelength (nm)	287	256	295	248
Linearity Range (µg/ml)	3-15	300-1500	3-15	300-1500
Regression equation (y = a + bc)	y = 0.0679x + 0.0118	y = 0.0009x + 0.0028	y = -0.0338x - 0.0056	y = 0.0004x + 0.0003
Slope (b)	0.0679x	0.0009x	-0.0338x	0.0004x
Intercept (a)	0.0118	0.0028	-0.0056	0.0003
Correlation Coefficient (r^2)	0.9995	0.9998	0.9996	0.9996
LOD (µg/ml)	0.1505	8.9063	0.1437	12.5117
LOQ (µg/ml)	0.4563	26.9888	0.4354	37.9143

Table 2: Statistical Validation Data for Accuracy Determination.

Level of % Recovery	Components	Amount present (µg/ml)	Amount of Standard drug added (µg)	Method A Total amount recovered (µg)	% Recovery	RSD	Method B Total amount recovered (µg)	% Recovery	RSD
80%	LEVO	6	4.8	10.79	99.90	0.3707	10.79	99.90	0.3707
	PPA	600	480	1079.04	99.91	0.3725	1079.04	99.91	0.3725
100%	LEVO	6	6	12.01	100.05	0.1272	12.01	100.05	0.1272
	PPA	600	600	1200.02	100.01	0.2701	1200.02	100.01	0.2701
120%	LEVO	6	7.2	13.19	99.92	0.1911	13.19	99.92	0.1911
	PPA	600	720	1318.03	99.85	0.1527	1318.03	99.85	0.1527

Table 3: Statistical Validation Data for Intra-day Precision.

Components	Method A		Method B	
	LEVO	PPA	LEVO	PPA
Mean	99.58	99.94	100.09	99.98
Standard Deviation	0.3573	0.4455	0.3643	0.0229

Relative Standard Deviation	0.3587	0.4474	0.3640	0.0229
Standard Error	0.1459	0.1819	0.1487	0.0093

n*=6

Table 4: Statistical Validation Data for Inter-day Precision.

Components	Method A		Method B	
	LEVO	PPA	LEVO	PPA
Mean	100.17	99.81	100.06	100.01
Standard Deviation	0.3582	0.3575	0.3505	0.0528
Relative Standard Deviation	0.3749	0.3755	0.3503	0.0528
Standard Error	0.1463	0.1530	0.1431	0.0215

n*=3

Accuracy

Accuracy studies were done as percent recovery, it was performed by adding constant amount of the standard drug to the sample taken from formulations at levels of 80%, 100% and 120% of the test concentration. The results are tabulated in Table no 2.

Precision

The Intraday and Interday precisions of the proposed spectrophotometric methods were determined by estimating the corresponding responses three times on the same day and on 3 different days over a period of one week for 3 different concentration and 3 replicates LEVO and PPA and the reported in terms of relative standard deviation (RSD). Statistical validation of data for Intraday and Interday precision methods as shown in Table no 3 and Table no 4.

LOD and LOQ

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision. In this study, LOD and LOQ were determined based on the standard deviation of the response and the slope of the corresponding curve using the following equations.

LOD = 3.3 SD/Slope and LOQ = 10 SD/Slope.

Where, SD is the standard deviation of the absorbance of the sample and the slope of the related calibrations curve.

RESULT AND DISSCUSION:

The selected drugs Levofloxacin and Phenylpropanolamine in Bulk and Formulation were estimated by using both simultaneous equation method and first order derivatives of UV spectrophotometric methods as per ICH guidelines. The methods were validated for all validation parameters as per ICH guidelines. The linearity range in both methods for LEVO and PPA was 3-15µg/ml and 300-1500µg/ml respectively. The % RSD for intraday and inter-day precision was found to be less than 2%. The methods have been validated in assay of active pharmaceutical ingredients. The accuracy of the methods was validated by recovery studies and was found to be significant and under specification limits, with % recovery 99-100%. The assay results were found to be within the acceptable limits.

CONCULSION:

The developed simultaneous equation method and first order derivative methods were found to be simple, precise, specific, and accurate and can be used for routine analysis of Levofloxacin and Phenylpropanolamine. Both methods were validated as per ICH guidelines.

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